A Multiple-Site, Phase 1/2, Safety and Efficacy Trial of a Recombinant Adeno-associated Virus Vector Expressing Retinoschisin (rAAV2tYF-CB-hRS1) in Patients with X-linked Retinoschisis

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Statement of Compliance

The study will be carried out in *co*mpliance with the protocol, the International Conference on Harmonisation Good Clinical Practice E6 guidelines (ICH GCP) and the United States Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR 46 and 21 CFR parts 50, 56 and 312).

All personnel involved in conducting this study will be required to provide evidence of GCP training and education on the protection of human research participants prior to study initiation.

DO NOT IMPLEMENT THIS PROTOCOL UNLESS SIGNED BY THE PRINCIPAL INVESTIGATOR ON THE SIGNATURE PAGE PROVIDED AS A SITE-SPECIFIC APPENDIX TO THIS PROTOCOL.

This protocol is a CONFIDENTIAL communication and is prepared for the sole purpose of informing investigators and their professional assistants who are participating in clinical trials.

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List of Abbreviations

AAV	adeno-associated virus
AE	adverse event/adverse experience
AGTC	Applied Genetic Technologies Corporation
ALT	alanine aminotransferase
BCVA	best corrected visual acuity
C	Celsius
cDNA	DNA complementary to RNA
CFR	Code of Federal Regulations
cGMP	current Good Manufacturing Practices
CRA	Clinical Research Associate
CTCAE	Common Terminology Criteria for Adverse Events
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
DSMC	Data and Safety Monitoring Committee
eCRF	electronic case report form
ETDRS	Early Treatment of Diabetic Retinopathy Study
ERG	electroretinogram
EVA	electronic visual acuity testing
EZ	ellipsoid zone
FDA	US Food and Drug Administration
ffERG	full-field ERG
GATE	German Adaptive Thresholding Estimation
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
ILM	inner limiting membrane
IND	Investigational New Drug Application
IOP	intraocular pressure
IRB	Institutional Review Board
ISCEV	International Society for Clinical Electrophysiology of Vision
ITR	inverted terminal repeat
kDa	kilodalton
LCA	Leber congenital amaurosis
MedDRA©	Medical Dictionary for Regulatory Activities
mfERG	multifocal ERG
mL	milliliter(s)
MTD	maximum tolerated dose
NIH	National Institutes of Health
OCT	optical coherence tomography
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PT	prothrombin time
PTT	partial thromboplastin time
rAAV	recombinant adeno-associated virus vector
rAAV2tYF-CB-hRS1	rAAV vector expressing retinoschisin

RNA	ribonucleic acid
RS1	retinoschisin (protein or gene)
SAE	serious adverse event/serious adverse experience
SD-OCT	spectral domain OCT
SKP	semi-automated kinetic perimetry
VFQ-25	25-Item National Eye Institute Visual Function Questionnaire
vg	vector genome(s)
VP1, VP2, VP3	structural proteins of AAV
XLRS	X-linked retinoschisis
YF	tyrosine phenylalanine
yr	years

Protocol Summary

Title: A Multiple-Site, Phase 1/2, Safety and Efficacy Trial of a Recombinant Adeno-associated Virus Vector Expressing Retinoschisin (rAAV2tYF-CB-hRS1) in Patients with X-linked Retinoschisis

Phase: 1/2

Population: Approximately 27 males with X-linked retinoschisis

Study Duration: Enrollment in this study is anticipated to take approximately 30 months. Enrolled participants will have frequent follow-up visits during the first year after study agent administration. To monitor for delayed adverse events (AEs) and assess the duration of any visual changes that occur, participants will be followed annually for an additional 4 years after the Month 12 visit.

Study Sites: This will be a multi-center study

Description of Study Agent: rAAV2tYF-CB-hRS1 is a replication-incompetent, recombinant adeno-associated virus (rAAV) vector that expresses the retinoschisin (RS1) protein after the vector enters retinal cells. This type of vector has been shown to improve visual function in animal models of X-linked retinoschisis (XLRS).

The rAAV2tYF-CB-hRS1 vector consists of a single-stranded DNA contained within an AAV capsid. The DNA contains the *RS1* cDNA in an expression cassette, flanked by inverted terminal repeat sequences that enable packaging of the DNA into AAV capsids. The AAV capsid was selected based on its ability to enter retinal cells when delivered by intravitreal injection. The rAAV2tYF-CB-hRS1 vector is produced using a replication-defective herpes simplex virus helper system in baby hamster kidney cells and purified by column chromatography. It is stored frozen and will be thawed and diluted to the appropriate concentration immediately before administration by the intravitreal route.

Objectives:

Primary: To evaluate the safety of rAAV2tYF-CB-hRS1 in patients with X-linked retinoschisis

Secondary: To evaluate the efficacy of rAAV2tYF-CB-hRS1 in patients with X-linked retinoschisis

Study Design: This will be a non-randomized, open label, Phase 1/2 dose escalation study. Approximately 27 participants will receive rAAV2tYF-CB-hRS1 by intravitreal injection in one eye on a single occasion as outlined in the schematic below.

Schematic of Study Design:

Group [a]	Age (yr)	Number of Subjects	Dose level		
			vg/mL	Volume	vg per eye
1A	≥ 18	3	1.43×10^{12}	0.07 mL	1×10^{11}
1B	≥ 18	3	1.43×10^{12}	0.07 mL	1×10^{11}
2	≥ 18	3	4.3×10^{12}	0.07 mL	3×10^{11}
2A	6-17	Up to 6	4.3×10^{12}	0.07 mL	3×10^{11}
3	≥ 18	3	4.3×10^{12}	0.14 mL	6×10^{11}
4	≥ 6	Up to 15	MTD [b]	MTD	MTD

[a] Visual acuity not better than 58 ETDRS letter score in Group 1A, 63 in Groups 1B, 2, 2A & 3, 68 in Group 4.

[b] MTD = maximum tolerated dose determined in Groups 1A, 1B, 2 and 3 for adults, in Groups 1A, 1B, 2, and 3 (adult participants only) and 2A (pediatric participants only) for pediatric participants.

Study Design (cont.):

Participants in Groups 1A, 1B, 2 and 3 will be at least 18 years of age and receive the vector at a lower dose (Groups 1A and 1B), middle dose (Group 2) or higher dose (Group 3). Participants in Group 1A will have visual acuity that is worse than participants in subsequent groups. Participants in Group 2A will be 6-17 years of age and receive the vector at the middle dose level. Participants in Group 4 will be at least 6 years of age and receive the vector at the maximum tolerated dose (MTD) determined in Groups 1A, 1B, 2 and 3 for adults; in Groups 1A, 1B, 2, and 3 (adult participants only) and 2A (pediatric participants only) for pediatric participants.

Enrollment will begin with Group 1A and will proceed to subsequent groups after review of safety data by a Data and Safety Monitoring Committee (DSMC). After review of safety data from Group 1A, participants will be enrolled in Groups 1B and 2 concurrently. After review of safety data from Groups 1A, 1B and 2, participants will be enrolled in Group 3. After review of safety data from Groups 1A, 1B, 2 and 3, participants ≥18 years of age will be enrolled in Group 4. As ocular inflammation has been seen in the first 12 subjects enrolled in the study and pediatric participants might be more prone to inflammation, pediatric participants (age 6-17) in this study will first be treated at the middle dose (Group 2A) prior to the enrollment in Group 4.

Safety will be monitored by evaluation of ocular and non-ocular AEs, hematology and clinical chemistry parameters and immune responses to RS1. Efficacy will be measured by evaluation of visual acuity, visual fields, microperimetry, contrast sensitivity, reading speed, optical coherence

tomography (OCT) with infrared montage, to include volumetric analysis of schisis cavity size, electroretinography (ERG), fundus photography and quality of life questionnaires. Other data collected will include immune responses to AAV and the presence of vector DNA in blood.

1 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Background Information

1.1.1 X-linked Retinoschisis

X-linked retinoschisis (XLRS), also known as X-linked juvenile retinoschisis, is an early onset retinal degenerative disease and is the leading cause of juvenile macular degeneration in males. Characteristic features include mild to severe loss in central vision, radial streaks arising from foveal schisis, splitting of inner retinal layers in the peripheral retina, and a negative electroretinogram (ERG) arising from a marked reduction in b-wave amplitude [1, 2]. Best corrected visual acuity is reduced to 20/100 or worse in most patients [3]. Disease progression and severity is highly variable even within families. During the course of the disease, secondary complications including retinal detachment and vitreous hemorrhage can occur, leading to a poor outcome. Female carriers are asymptomatic although detailed clinical examination can reveal minor retinal abnormalities [4].

XLRS is caused by mutations in the gene that encodes a protein called retinoschisin (RS1), a cell-surface adhesion molecule expressed by photoreceptor and bipolar cells of the retina [3]. The 24-kDa protein has two conserved sequence motifs; an initial signal sequence targets the protein for secretion and the larger discoidin domain is implicated in cell adhesion. RS1 helps to maintain the structural organization of the retinal cell layers and promotes visual signal transduction.

There is no specific treatment for XLRS. Anecdotal reports suggest that topical carbonic anhydrase inhibitors may provide some reduction in degree of schisis detected by OCT and improvement in visual acuity in some but not all patients [5-9], but the absence of controlled clinical trials makes interpretation of these reports difficult. No products have been approved by regulatory agencies for treatment of this condition.

Mice deficient in retinoschisin have been developed and used to obtain insight into the role of retinoschisin in retinal structure, function and pathology. Studies in these murine models of XLRS have shown that recombinant adeno-associated virus (rAAV) gene therapy vectors expressing normal RS1 can provide significant restoration of retinal structure and function in RS1-deficient mice [10-16]. These studies support the medical plausibility that intraocular delivery of a rAAV vector expressing human RS1 in patients with XLRS will also provide benefit to these patients.

1.1.1.1 Adeno-associated Virus

Adeno-associated virus (AAV) is a parvovirus with a single-stranded DNA genome [17]. It was originally discovered as a laboratory contaminant of adenovirus cultures [18, 19] and it requires a helper virus such as adenovirus or herpesvirus to complete its replication cycle in culture and in humans [20, 21]. Although serologic tests indicate a high prevalence of prior infection, AAV has not been associated with human disease [22].

The AAV virion is composed of a mixture of three structural proteins (VP1, VP2 and VP3) which encapsidate the linear, single-strand DNA molecule [17]. The genome contains approximately 4700 nucleotides consisting of inverted terminal repeat (ITR) sequences of 145 nucleotides that flank the two viral genes, *rep* and *cap*. The *rep* gene is transcribed from two promoters, each of which generates spliced and unspliced transcripts that are translated into the four replication proteins. The *cap* gene uses a separate promoter to generate transcripts for the three structural proteins through a combination of alternate splicing and alternate translation start codons. The three structural proteins self-assemble to form an icosahedral capsid in which the AAV genomic DNA is contained.

Replication of AAV DNA occurs via a single-strand displacement mechanism [23-25] that is dependent on the AAV Rep proteins, host proteins and additional proteins provided by a helper virus. In the absence of the helper virus, wild-type AAV can establish latency in cell culture by largely site-specific integration with the assistance of Rep proteins through the interaction of the ITR with the chromosome [26]. AAV does not appear to integrate in humans, but instead exists as circular double-stranded episomes within the nucleus but separate from host chromosomal DNA [27].

1.1.2 Recombinant AAV Vectors

Recombinant AAV (rAAV) vectors have been developed by deleting the viral *rep* and *cap* genes, inserting a transgene expression cassette between the ITRs, and packaging the vector DNA into AAV capsids in a packaging cell. rAAV vectors are uniquely suitable for *in vivo* gene therapy because they are non-toxic, highly efficient at transducing a wide variety of non-dividing cell types, and persist for long periods, primarily in episomal form, resulting in long-term expression of the transgene [28]. rAAV vectors have been effective for treatment of a wide variety of animal models of genetic diseases, including retinal diseases [11, 12, 29-32], hemophilia [33, 34], muscular dystrophy [35, 36], lysosomal storage disorders [37-39] and diseases of the central nervous system [40-42].

Several serotypes of AAV have been identified in humans and a larger number have been identified in nonhuman primates [43]. Hybrid AAV vectors can be prepared that cross-package rAAV DNA containing serotype 2 ITRs into the capsids of other AAV serotypes [44], and these hybrid vectors have different physical characteristics, including different affinities for the cell surface receptors to which they bind and different tissue tropism *in vivo*.

Tropism can also be modified by selective mutations of the AAV capsid gene. Capsids with mutations in surface-exposed tyrosine residues to phenylalanine (YF mutants) have been documented to enhance the efficiency of transduction in mouse retina after intravitreal injection, and several of these rAAV mutants were found to display strong and widespread transgene expression in many retinal cells after subretinal or intravitreal delivery compared with their wild-type counterparts [45]. Subsequent studies in mice showed that intravitreal injection of rAAV-GFP vectors packaged in AAV2 capsids containing multiple YF mutations achieved uniform GFP expression through the entire thickness of the retina [46].

For intravitreal injection, an important consideration is the different anatomy of the primate eye and the eye of lower animals. Humans and nonhuman primates are the only species that have cone photoreceptors, which are responsible for fine visual acuity and color vision, concentrated in the central regions of the macula and especially the very central fovea. They also have an inner limiting membrane (ILM) that is thicker than the ILM in mice or dogs, except in the region of the fovea where the ILM is much thinner. Thus intravitreal injection of rAAV vectors of a variety of unmodified serotypes can cross the ILM in mice but have little or no ability to cross the ILM in nonhuman primates except in the region around the macula. Studies in rhesus macaques described in section 1.1.4.2 below showed that intravitreal injection of rAAV vectors packaged in AAV2 capsids containing three YF mutations (AAV2tYF) is able to achieve efficient transduction of retinal ganglion cells and foveal cone photoreceptors and secretion and spread of retinoschisin throughout the inner retinal layers in the macula, which is a critical area where schisis occurs in patients with XLRS.

1.1.3 Human Clinical Experience with Recombinant AAV Vectors

rAAV vectors have previously demonstrated their utility in the development of gene-based therapies [47, 48] and through June 2014 have been evaluated in 117 clinical trials, of which 52 were for monogenic diseases [49]. Clinical trials for ocular diseases include Leber congenital amaurosis [50-52] and choroideremia [53], in which AAV vectors were administered by subretinal injection, and age-related macular degeneration, in which AAV vectors were administered by subretinal injection [54] or intravitreal injection [55]. Available data from these studies have reported no serious adverse events (SAEs) attributed to the AAV vector and evidence of improvement in one or more measures of visual function in the majority of patients.

Results of initial human clinical experience with rAAV2tYF-CB-hRS1 are summarized in the Clinical Investigators Brochure.

1.1.4 Nonclinical Studies with AAV-RS1 Vectors

1.1.4.1 Efficacy Studies in RS1-deficient Mice

Studies in animal models of XLRS indicate that rAAV gene therapy vectors expressing normal mouse or human RS1 can correct the defects in RS1 deficient mice [10-16]. The vectors used in these studies are summarized in Table 1.

Table 1 Vectors used in proof-of-concept studies in RS1 deficient mice

AAV serotype	cDNA	Promoter	Route of administration	References
2	Mouse RS1	CMV	Intravitreal	[10-14]
5	Human RS1	mOP500	Subretinal	[11-13]
8	Human RS1	hRS1	Intravitreal	[15]
7m8	Human RS1	rho or CAG	Intravitreal	[16]

CMV = cytomegalovirus, RS1 = retinoschisin, hRS1 = human RS1, mOP500 = 500 base pair mouse opsin, rho = rhodopsin, CAG = CMV enhancer / chicken beta actin (synonymous with CBA or CB)

Each of these vectors was shown to restore the reduced ERG b-wave response towards normal in vector-treated eyes compared to control eyes. Several studies also demonstrated preservation of photoreceptor structure, reduction in the number and size of schisis cavities, and improvement in visually mediated behavior. Rescue of the XLRS phenotype was demonstrated using mouse or human RS1 cDNA, a variety of promoters, and either subretinal or intravitreal delivery.

One of these studies [15] indicated that AAV transduction efficiency of the retinal layers differs significantly between wild-type mice and RS1-deficient mice. As stated in a report of that study, “the absence of retinoschisin protein apparently causes a striking change in the properties of the retina. Wild-type murine retinas are not permeable to AAV vectors. This lack of permeability is not dependent on AAV serotype … Vectors made from all serotypes tested so far are able to penetrate all retinal layers of Rs1-KO mice and will even transduce the retinal pigment epithelium. The lack of retinoschisin seems to significantly change the properties of the interstitial matrix making the retina permeable to particles that are at least 28 nm in diameter. Retinal permeability to AAV vectors is uniform across the Rs1-KO retina and is not localized to regions showing schisis pathology.” If a similar phenomenon occurs in patients with XLRS, intravitreal delivery of an AAV-RS1 vector is expected to penetrate more deeply into the outer retinal layers of such patients than it would in individuals who do not have XLRS.

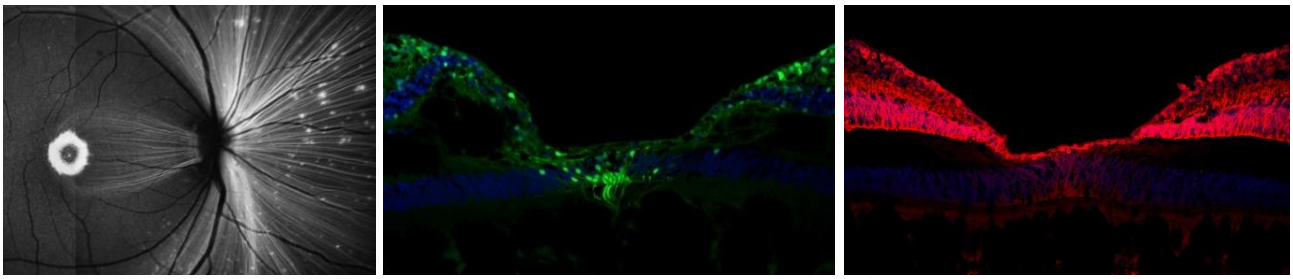
Another study compared AAV-RS1 vectors containing either a rhodopsin promoter that restricts transgene expression to rod photoreceptors or a CAG promoter that achieves efficient

transgene expression in all cell types and showed efficient rescue of ERG b-wave responses with both promoters [16].

1.1.4.2 Vector Targeting Studies in Nonhuman Primates

A study was conducted in rhesus macaques comparing rAAV vectors expressing GFP or RS1, driven by a CB promoter and packaged in each of three types of AAV capsids, for their ability to transduce the retina after intravitreal injection. Because the amino acid sequence of human and rhesus are identical, the RS1 cDNA contained a 10 amino acid "myc" tag that is recognized by an anti-myc monoclonal antibody. All three capsid serotypes evaluated were able to penetrate the inner limiting membrane after intravitreal injection and achieve significant expression of GFP or RS1$_{myc}$ in the retinal ganglion cell ring and foveal cone photoreceptors. The pattern of RS1$_{myc}$ expression in the macula was essentially the same as that of endogenous RS1, except RS1$_{myc}$ did not penetrate efficiently to the photoreceptor inner segments in areas outside the fovea. Less intense expression of GFP or RS1$_{myc}$ was seen in the peripheral retina, but the pattern of RS1$_{myc}$ in the periphery was consistent with the expected patter for a protein secreted into the extracellular matrix. The most consistent results were obtained with an AAV2 capsid containing tyrosine to phenylalanine (YF) mutations in three surface-exposed tyrosine residues (AAV2tYF). An example of expression of GFP and RS1$_{myc}$ after intravitreal injection with vectors packaged in the AAV2tYF is shown in Figure 1.

Figure 1 Expression of GFP or RS1$_{myc}$ in nonhuman primate retina



A rhesus macaque received an intravitreal injection of rAAV2tYF-CB-GFP in one eye and rAAV2tYF-RS1$_{myc}$ in the other eye at a dose of 1×10^{11} vg/eye. An in-life fundus fluorescence photo of an eye injected with the GFP vector (left panel) showed expression of GFP (white) in the macula (donut-shaped area) and fovea (dot in the center of the macula), retinal nerve fibers, and scattered foci in the peripheral retina. Immunohistochemical examination of the same eye (middle panel) showed expression of GFP (green), which is an intracellular protein, in retinal ganglion cells across the macular region of the retina and in foveal cones. Immunohistochemical examination of the other eye (right panel) showed expression of RS1$_{myc}$ (red), demonstrating that the protein was secreted into the extracellular matrix and spread through the inner layers of the retina.

These results indicate that AAV vectors with a rAAV2tYF capsid and CB promoter are able to transduce appropriate retinal cells to enable delivery of RS1 protein to the region of the retina that is most affected in patients with XLRS.

1.1.4.3 Nonclinical Safety and Biodistribution Studies

Safety and biodistribution studies evaluating rAAV2tYF-CB-hRS1 have been conducted in RS1-deficient mice and normal cynomolgus macaques.

1.1.4.3.1. Safety and biodistribution in RS1-deficient mice

In the mouse study, three groups of male mice (n = 20 per group) each received an intravitreal injection of 1 µL containing rAAV2tYF-CB-hRS1 at a concentration of 1 x 10^{12} vg/mL (1 x 10^{9} vg per eye) or 4 x 10^{12} vg/mL (4 x 10^{9} vg per eye) or 1 µL of vehicle control. Half the animals were sacrificed 30 days after vector administration and the remaining animals were sacrificed 90 days after vector administration. At each sacrifice time point half the animals were used for evaluation of safety and half were used for evaluation of biodistribution.

The intravitreal injection procedure was well tolerated in all groups. Minimally to mildly higher white blood cell and absolute lymphocyte and monocyte counts at Day 90 in two animals in the low dose vector group were suggestive of mild inflammation. There were no other intergroup differences in hematology or clinical chemistry analyses and no test article-related gross necropsy observations.

Microscopic pathology results demonstrated minimal to slight mononuclear cell infiltrate and minimal to moderate RS1 labelling of the retina in both vector-treated groups. At the Day 90 sacrifice there was a decrease in the severity of splitting/disorganization of the inner nuclear layer of the retina in high-dose vector-treated animals. Results of biodistribution testing detected vector DNA in the injected eye but in no other tissue.

1.1.4.3.2. Safety and biodistribution in cynomolgus macaques

In the nonhuman primate study, three groups of male macaques (n = 6 per group) each received an intravitreal injection of 110 µL containing rAAV2tYF-CB-hRS1 at a concentration of 3.6 x 10^{11} vg/mL (4 x 10^{10} vg per eye) or 3.6 x 10^{12} vg/mL (4 x 10^{11} vg per eye) or 110 µL of vehicle control. Half the animals were sacrificed 14 days after vector administration and the remaining animals were sacrificed 90 days after vector administration.

The intravitreal injection procedure was well tolerated in all groups. Ocular exams demonstrated varying levels of inflammation over time.

In the vehicle control group, aqueous cells ranged from trace to 4+ at 1 week after injection and resolved by Week 2. Vitreous cells ranged from trace to 2+ at 2 to 3 weeks after injection, resolved by Week 4 in four animals and in the other two animals were resolved or resolving at Week 12. None of the animals in this group had vitreous haze.

Ocular inflammation was greater in the vector-treated animals and was more pronounced at the higher dose. Aqueous cells ranged from 1+ to 4+ at Week 1 in all animals, resolved by Week 2, recurred at Week 2 to 4 in two animals in the low dose and three animals in the high dose group, and then resolved over the next several weeks. Vitreous cells developed in 5 of 6 animals in the low dose and all animals in the high dose group, and persisted at 2+ or greater for more than 4 weeks in two animals in the low dose and three animals in the high dose group. Vitreous haze developed at Week 4 in one animal in the low dose and three animals in the high dose group and resolved over the next several weeks.

The frequency, magnitude and time course of ocular inflammation at doses of 4×10^{10} and 4×10^{11} vg/eye in this study was similar to that seen in a toxicology study of an AAV2 vector expressing a vascular endothelial growth factor receptor inhibitor delivered by intravitreal injection at a dose of 2.4×10^{10} vg/eye [56]. When that product was evaluated by intravitreal injection in a human clinical trial, ocular inflammation developed in 1 of 11 subjects who received the 2.4×10^{10} vg dose, which was treated with topical steroids for 5 weeks during which time it resolved [57].

There was no apparent effect of vehicle control or rAAV2tYF-CB-hRS1 at either dose level on intraocular pressure, and no changes in ERG or visual evoked potential responses.

There were no intergroup differences in hematology, coagulation or clinical chemistry parameters and no test article-related gross necropsy observations.

Microscopic pathology results demonstrated RS1 labelling of the ganglion cell layer at the foveal slope and minimal or moderate mononuclear infiltrate at the optic disc and/or around blood vessels in the ganglion cell layer, iris, and ciliary body of the injected eye at both dose levels. RS1 labelling of the ganglion cell layer at the foveal slope was observed in all animals at the higher dose and in 5 of 6 animals at the lower dose. Mononuclear infiltrates at the optic disc and/or around blood vessels in the ganglion cell layer, iris, and ciliary body of the injected eye was observed in 2 of 6 animals at the lower dose and 4 of 6 animals at the higher dose. Results of biodistribution testing detected vector DNA in the injected eye but minimal or no vector DNA was found in any other tissue.

1.2 Rationale

1.2.1 Rationale for Gene Therapy of XLRS

XLRS is selected for experimental treatment by means of gene transfer based on substantial preclinical evidence demonstrating the ability of AAV vectors expressing RS1 to achieve improvement in disease manifestations in RS1-deficient mice, an acceptable safety profile in mice and nonhuman primates, and clinical experience indicating that AAV vectors expressing a variety of transgenes can be administered safely in human patients with preliminary evidence of efficacy.

The retinas of patients with XLRS are more fragile than normal and prone to disease complications, such as vitreous hemorrhage and retinal detachment, which may lead to severe visual impairment [58]. Therefore, a subretinal injection procedure in this patient population may pose a higher risk to the visual function of the individual, and intravitreal delivery is preferred. Studies in nonhuman primates demonstrated that intravitreal injection of rAAV2tYF-CB-hRS1 achieved expression of RS1 protein in the macula and fovea, indicating the potential to deliver RS1 protein to the region of the retina that is most affected in patients with XLRS. Studies in RS1-deficient mice indicate that the absence of retinoschisin changes the permeability of the retina to AAV vectors [15], suggesting that a rAAV2tYF-CB-hRS1 vector may be even more effective at delivering normal RS1 protein to larger areas of the retina in patients with XLRS.

1.2.2 Rationale for Dosage Level

Administration of rAAV2tYF-CB-hRS1$_{myc}$ at a dose of 1×10^{11} vg per eye by intravitreal injection in nonhuman primates demonstrated expression of RS1$_{myc}$ protein in the macula and fovea. Administration of rAAV2tYF-CB-hRS1 at a dose of 4×10^{10} or 4×10^{11} vg per eye by intravitreal injection in nonhuman primates demonstrated ocular inflammation that was maximal 4 weeks after administration and progressively improved over the ensuing 2 months. At sacrifice, microscopic pathology results demonstrated RS1 labelling of the ganglion cell layer at the foveal slope in 5 of 6 animals in the lower dose group and all animals in the higher dose group. Microscopically evident inflammatory cells, usually minimal and occasionally moderate in intensity, were seen in 2 of 6 injected eyes in the lower dose group and 4 of 6 injected eyes in the higher dose group. The volume of a human eye is approximately 5 mL and the volume of a nonhuman primate eye is approximately 2.5 mL. Based on this information, it is concluded that dose levels between 1×10^{11} vg per eye and 6×10^{11} vg per eye are predicted to have the potential for providing benefit with an acceptable safety profile.

Administering the dose in a volume of 70 µL or 140 µL is considered to be an acceptable volume in human eyes, and is in line with the 110 µL volume used in nonhuman primate studies with no AEs related to the injection volume.

The dose escalation schedule is typical of Phase 1 trials of gene therapy products for orphan diseases. Enrolling subjects with the most advanced disease in Group 1A will provide a favorable risk-benefit profile for the product and study procedure. If the safety evaluations identify no dose-limiting toxicity in Group 1A, subjects with less severe disease will be appropriate for enrollment at the next higher dose (Group 2). Subjects with less severe disease will also be enrolled in Group 1B, at the same dose level as Group 1A, in order to increase the ability to detect evidence of efficacy at this dose level. If the safety evaluations identify no dose-limiting toxicity in Group 2, subjects with less severe disease will be appropriate for enrollment at the highest dose level (Group 3). If the safety evaluations identify no dose-limiting toxicity in Group 2A, it will be appropriate to enroll subjects in Group 4.

1.2.3 Rationale for Inclusion of Pediatric Patients

XLRS is a congenital condition present from birth, there is no approved alternative therapy, and results in RS1-deficient mice have shown that the best improvements in visual function occur in younger animals [13]. This information provides the rationale that pediatric patients with XLRS are likely to benefit from participation in this clinical trial.

1.3 Potential Risks and Benefits

1.3.1 Potential Risks

Risks associated with the study include risks related to the intravitreal injection procedure used to deliver the study agent, risks related to the study agent, risks related to prophylactic and post-study agent administration medications, and risks related to other study procedures.

Human clinical experience with intravitreal administration of rAAV vectors is limited, so the risks associated with this clinical trial are not completely known. Based on preclinical studies with rAAV2tYF-CB-hRS1 in RS1-deficient mice and nonhuman primates, it is anticipated that intravitreal administration of rAAV2tYF-CB-hRS1 will not cause SAEs in humans. The safety of rAAV vectors in humans is also supported by limited clinical experience with subretinal and intravitreal injection of AAV vectors expressing other transgenes, and extensive experience with rAAV vectors administered by other routes of administration (see Section 1.1.3).

Risks associated with anesthesia are infrequent but may include allergic reactions to local or general anesthetic drugs and a low risk of penetration of the eye or optic nerve during injection of local anesthetic drugs. General anesthesia may also be associated with nausea or systemic hypotension, and rarely with prolonged unconsciousness, drowsiness or disorientation, tachycardia or dysrhythmias, pneumonia or ,atelectasis, or death.

Risks related to the study agent include inflammatory responses in the eye, which can manifest as pain, redness or swelling. The study agent and/or any resulting inflammation may lead to changes in the vitreous, posterior vitreous detachment (PVD), traction on the retina, retinal breaks (holes and tears), and retinal detachments. Inflammation may involve any ocular tissue, including the vitreous, the optic nerve, and the retina. Any of these events may require laser therapy (photocoagulation), cryotherapy, and/or surgical repair. Steroids may be used as prophylactic treatment for inflammation in this study as described in the Study Manual of Procedures. Risks associated with topical, subconjunctival, intravitreal or subtenon steroid use includes blurred vision, temporary burning or stinging, itching, eye pain. The most common risks associated with topical/local steroids increased intraocular pressure, corneal infections, and cataract formation.

Side effects associated with oral steroid are multisystemic and are related to both the average dose and duration of treatment. Major side effects include hypothalamic-pituitary-adrenal axis

suppression, psychiatric and sleep disturbances, weight gain and cushingoid appearance, heightened risk of common and opportunistic infections, facial & body hair growth, numbness/tingling in the legs and arms, shortness of breath, swelling of the limbs, flushed dry skin, hypertension, diabetes mellitus and worsening of glycemic control in patients with existing diabetes, gastrointestinal side effects, including gastritis and peptic ulcer disease, decreased bone density, osteoporosis, myopathy, elevated intraocular pressure/glaucoma, cataract formation, skin thinning and purpura and isolated leukocytosis. These side effects are generally associated with long-term steroid use. Prolonged systemic use of steroid in children may result in growth retardation.

There is a potential for vector spread to the bloodstream. In clinical trials with AAV vectors administered by subretinal injection, the frequency of this was low and lasted for less than a few weeks after vector administration. Given the low doses to be administered and the low risk of vector spread to the bloodstream, vector spread to gonads is not considered to be a reasonable possibility, and no precautions related to a participant's partner becoming pregnant will be recommended.

There is a potential risk that vector DNA sequences could integrate into a host chromosome and result in insertional mutagenesis, but studies in animals indicate this is very unlikely to occur.

It is likely that subjects will develop antibodies to AAV, which might make it difficult or impossible for them to receive treatment with AAV vectors in the future.

There is a potential risk that subjects will develop an immune response to RS1 protein, and the clinical consequences if this were to occur are unknown.

Risks associated with intravitreal injection of any product include the potential for pain, subconjunctival or vitreous hemorrhage, increased intraocular pressure, infection, inflammation, retinal holes, retinal tears, retinal detachments or post-surgical cataract development that could require additional eye surgery. Infection occurs uncommonly after intravitreal injections and can usually be treated with antibiotics. All of these complications could result in worsening of vision. There may also be an increase in intraocular pressure that would require treatment to lower the pressure.

Risks associated with anterior chamber tap (paracentesis), if preferred to be performed by the Investigator before the injection of the high dose, include potential for pain, subconjunctival hemorrhage, infection, iris trauma, entry site leak, hypotony, hyphaema or endophthalmitis.

Risks associated with other study procedures include redness, discomfort or allergic reactions to topical medications used to dilate the pupil prior to visual function tests. High blood pressure, cardiac dysrhythmias and closed angle glaucoma may be exacerbated by some of these medications, and light sensitivity may be experienced while the pupil is dilated. Corneal

abrasions may result from the contact lenses used in performing electroretinography testing. Risks associated with blood drawing include local pain/discomfort, bruising, infection or fainting.

There may also be other risks that are currently unknown.

1.3.2 Adequacy of Protection from Potential Risks

The medical history and physical examination performed during the screening and baseline visits will identify individuals with ophthalmologic or other medical conditions that would increase the risks associated with participation in the study. Results of all laboratory and safety evaluations will be reviewed by the investigator and sponsor throughout the trial, and the DSMC will review safety data from completed dosage level groups before enrollment into subsequent dosage level groups (see Section 7.4).

Intravitreal injection of the study agent will be performed by experienced personnel with strong attention paid to aseptic technique to avoid the risks listed above. Participants will be followed up at frequent study visits with clinical evaluation and ophthalmic examination to prevent and/or mitigate the risks associated with steroid use.

Participants will be provided with telephone numbers to contact the investigators for any questions or concerns about possible post-procedure complications or side effects from study agent administration, and will be promptly evaluated and treated if necessary to minimize the consequences of any complications.

To minimize the likelihood of pain and bruising, phlebotomy will be performed by experienced phlebotomists.

1.3.3 Provisions for Injury

In the event of injury resulting from procedures associated with the clinical trial, professional medical treatment will be provided to the participants at the clinical trial site where the clinical trial procedures were performed.

1.3.4 Potential Benefits

Based on the available preclinical efficacy data, there is a potential that intravitreal injection of rAAV2tYF-CB-hRS1 may provide some degree of improvement in visual function in some of the individuals participating in this study. However, the degree of any benefit that may occur is unknown.

In addition, results of this study will provide important information that will assist in designing future studies of intravitreal gene delivery to treat blinding diseases.

1.3.5 Risk/Benefit Analysis

There is no currently approved treatment for XLRS. Preclinical studies indicate that intravitreal injection of rAAV2tYF-CB-hRS1 offers a reasonable hope for improving vision in affected humans and have demonstrated an acceptable safety profile. Clinical studies with other AAV vectors administered to patients with several types of retinal disease have demonstrated preliminary evidence of efficacy and no SAEs related to the study agent. Thus the potential benefits are considered to outweigh the potential risks for participants in this clinical trial.

1.3.6 Protection of Pediatric Participants

FDA regulations in 21 CFR Part 50, Subpart D, specify that clinical investigation in which more than minimal risk to children is presented by an intervention that holds out the prospect of direct benefit for the individual subject may involve children as subjects only if (a) the risk is justified by the anticipated benefit to the subjects; (b) the relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches; and (c) adequate provisions are made for soliciting the assent of the children and permission of their parents or guardians. Information relevant to these requirements includes the following:

As stated above, preclinical studies indicate that intravitreal injection of rAAV2tYF-CB-hRS1 offers a reasonable hope for improving vision in affected humans and have demonstrated an acceptable safety profile. The clinical protocol design requires that the study agent be tested in adult participants before enrolling any pediatric participants, thereby reducing the risk of unexpected AEs that might occur if pediatric participants were treated in the absence of such information. Because there is no approved therapy for XLRS, the available alternative approach is to monitor the patient and treat complications such as vitreous hemorrhage or retinal detachment if they occur. As described in Section 6.1, the study provides for soliciting the assent of the children and permission of their parents or guardians to participate in the study.

Based on this information, protection of pediatric participants is considered to be compliant with FDA regulations.

2 OBJECTIVES

2.1 Study Objectives

- The primary objective of this study is to evaluate the safety of rAAV2tYF-CB-hRS1 in patients with X-linked retinoschisis.
- The secondary objective of this study is to evaluate the efficacy of rAAV2tYF-CB-hRS1 in patients with X-linked retinoschisis.

2.2 Outcome Measures

2.2.1 Safety Outcomes

- Number and proportion of participants experiencing ocular or non-ocular adverse events,
- Number and proportion of participants experiencing any abnormal hematology or clinical chemistry parameter,
- Changes in titers of serum antibodies or IFN-γ ELISPOT responses to RS1.

2.2.2 Efficacy Outcomes

- Changes in best corrected visual acuity,
- Changes in schisis detected by optical coherence tomography (OCT) with infrared montage, to include volumetric analysis of schisis cavity size,
- Changes in static and kinetic visual fields measured using the Octopus perimeter,
- Changes in visual fields measured by microperimetry,
- Changes in reading speed test,
- Changes in contrast sensitivity,
- Changes in full-field electroretinogram (ffERG),
- Changes multifocal electroretinogram (mfERG),
- Changes in quality of life questionnaire responses.

2.2.3 Other Outcomes

- Changes in fundus photographs,
- Changes in serum antibodies or IFN-γ ELISPOT responses to AAV,

- Number and proportion of participants with rAAV2tYF-CB-hRS1 vector DNA detectable in blood by PCR assay

3 STUDY DESIGN

This will be a non-randomized, open-label, Phase 1/2 dose escalation study of the safety and efficacy of rAAV2tYF-CB-hRS1 administered by intravitreal injection in one eye in individuals with XLRS. The primary study endpoint will be safety and the secondary study endpoint will be efficacy.

Each participant will receive rAAV2tYF-CB-hRS1 by intravitreal injection in one eye on a single occasion as outlined in the schematic below.

Schematic of Study Design:

Group [a]	Age (yr)	Number of subjects	Dose level		
			vg/mL	volume	vg per eye
1A	≥ 18	3	1.43×10^{12}	0.07 mL	1×10^{11}
1B	≥ 18	3	1.43×10^{12}	0.07 mL	1×10^{11}
2	≥ 18	3	4.3×10^{12}	0.07 mL	3×10^{11}
2A	6-17	Up to 6	4.3×10^{12}	0.07 mL	3×10^{11}
3	≥ 18	3	4.3×10^{12}	0.14 mL	6×10^{11}
4	≥ 6	Up to 15	MTD [b]	MTD	MTD

[a] Visual acuity not better than 58 ETDRS letter score in Group 1A, 63 in Groups 1B, 2, 2A, & 3, 68 in Group 4.

[b] MTD = maximum tolerated dose determined in Groups 1A, 1B, 2 and 3 for adults; in Groups 1A, 1B, 2, and 3 (adult participants only) and 2A (pediatric participants only) for pediatric participants.

Participants in Groups 1A, 1B, 2 and 3 will be at least 18 years of age and receive the vector at a lower dose (Groups 1A and 1B), middle dose (Group 2) or higher dose (Group 3). Participants in Group 1A will have visual acuity that is worse than participants in subsequent groups. Participants in Group 2A will be 6-17 years of age and receive the vector at the middle dose level. Participants in Group 4 will be at least 6 years of age and receive the vector at the maximum tolerated dose (MTD) determined in Groups 1A, 1B, 2 and 3 for adults; in Groups 1A, 1B, 2, and 3 (adult participants only) and 2A (pediatric participants only) for pediatric participants..

Participants in all groups will be selected from individuals who are not currently being treated with a carbonic anhydrase inhibitor and have not been treated with any carbonic anhydrase inhibitor within the 3 months prior to Day 0 (study agent administration). Participants will be asked not to begin treatment with a carbonic anhydrase inhibitor within 6 months after study agent administration.

Individuals who have been participating in the natural history study XLRS-001, entitled “Clinical Evaluation of Individuals with X-linked Retinoschisis (XLRS)”, may discontinue their participation in that study and be enrolled in the present study if all appropriate entry criteria are met. In this case, results of the evaluations from the most recent visit in the natural history study, excluding OCT, can be used as part of the appropriate screening visit evaluations for the present study, as long as all the evaluations were conducted according to the current Study Manual of Procedures and Reading Center Manual of Procedures, and the visit occurred no more than 3 months prior to the planned date of study agent administration. If the most recent visit occurred more than 3 months prior to the planned date of study agent administration, all screening visit examinations will be performed.

Enrollment will begin with Group 1A and will proceed to subsequent groups after review of safety data by the DSMC. After review of safety data from Group 1A, participants will be enrolled in Groups 1B and 2 concurrently. After review of safety data from Groups 1A, 1B and 2, participants will be enrolled in Group 3. After review of safety data from Groups 1A, 1B, 2 and 3, participants ≥18 years of age will be enrolled in Group 4. As ocular inflammation has been seen in the first 12 subjects enrolled in the study and pediatric participants might be more prone to inflammation, pediatric participants (age 6-17) in this study will first be treated at the middle dose (Group 2A) prior to the enrollment in Group 4.

Within groups 1A, 1B, 2, 2A, and 3, enrollment of participants will be staggered by at least 2 weeks to allow adequate time for review of safety information by the investigators and sponsor.

Within Group 4, enrollment of the first 3 pediatric participants will be staggered by at least 2 weeks to allow adequate time for review of safety information by the investigators and sponsor.

Safety will be monitored by evaluation of ocular and non-ocular AEs, hematology and clinical chemistry parameters and immune responses to RS1.

Efficacy will be measured by evaluation of visual acuity, visual fields, microperimetry, contrast sensitivity, reading speed, OCT with infrared montage, to include volumetric analysis of schisis cavity size, ERG, and quality of life questionnaires.

Other data collected will include fundus photography, immune responses to AAV and the presence of vector DNA in blood.

Approximately 27 participants will be enrolled in this study. Enrollment in this study is anticipated to take approximately 30 months. Enrolled participants will have frequent follow-up visits during the first year after study agent administration.

To monitor for delayed AEs and assess the duration of any changes in visual function or structure that occur, participants will be followed annually for an additional 4 years after the Month 12 visit.

4 STUDY POPULATION

Approximately 27 male individuals with X-linked retinoschisis will be enrolled in this study. Potential participants (and a parent or guardian of children <18 years of age) will be identified from a natural history study being conducted by the investigators or from the clinical practice of or referrals to the investigators. Prospective participants will be informed of the purpose, the possible risks and benefits, and other details of the study.

After potential participants sign an informed consent form, they will be evaluated by obtaining a medical history, performing a physical examination, and obtaining blood samples. Individuals who meet the inclusion and exclusion criteria will be enrolled into the study.

4.1 Subject Inclusion Criteria

Subjects must meet all of the following inclusion criteria to participate in this study.

1. Retinal disease consistent with a diagnosis of X-linked retinoschisis and documented mutations in the *RS1* gene (including null mutations and mutations that code for abnormal RS1 protein, except as specified in section 4.2),

2. Male individual at least 18 years of age, inclusive (Groups 1A, 1B, 2 and 3), 6-17 years of age, inclusive (Group 2A), or at least 6 years of age, inclusive (Group 4),

3. Able to perform tests of visual and retinal function,

4. Visual acuity in the study eye not better than:

    - Snellen equivalent 20/80 (58 ETDRS letter score) in the treated eye for Group 1A,

    - Snellen equivalent of 20/63 (63 ETDRS letter score) in the treated eye for Groups 1B, 2, 2A, and 3,

    - Snellen equivalent of 20/50 (68 ETDRS letter score) in the treated eye for Group 4,

    The eye with the worse visual acuity will be selected as the study eye. If both eyes have the same visual acuity, the choice of study eye will be determined at the discretion of the investigator in consultation with the participant. ETDRS letter score is the main VA inclusion criterion.

5. Not currently being treated with a carbonic anhydrase inhibitor and not treated with any carbonic anhydrase inhibitor within the 3 months prior to Day 0 (study agent administration).,

6. Have acceptable laboratory parameters:
    - Hemoglobin ≥ 12.0 g/dL,
    - White blood cell count 3,300 – 12,000 cells/mm^3,
    - Platelet count 125,000 – 550,000/mm^3,
    - Alanine aminotransferase (ALT) not higher than 1.5 times the upper limit of the normal range for study laboratory,
    - Serum creatinine not higher than 1.1 times the upper limit of the normal range for study laboratory,
    - Prothrombin time (PT) ≤ 14.5 seconds, inclusive, and partial thromboplastin time (PTT) ≤ 36.0 seconds,
7. Be willing to discontinue aspirin, aspirin-containing products, and any other drugs that may alter coagulation, at least 7 days prior to study agent administration.
8. Signed informed consent obtained before screening.

4.2 Subject Exclusion Criteria

All subjects meeting any of the following exclusion criteria prior to enrollment will be excluded from study participation:

1. Prior receipt of any AAV gene therapy product,
2. Any of the following mutations in the *RS1* gene: R141H, C59S or C223S,
3. Serum anti-AAV neutralizing antibody titer against AAV2tYF >1:20,
4. Pre-existing eye conditions that would contribute significantly to visual loss or increase the risk of an intravitreal injection (e.g. advanced glaucoma, uveitis or large retinal detachment),
5. Lens, cornea, or other media opacities that preclude adequate visualization and testing of the retina,
6. Use of anticoagulants or anti-platelet agents within 7 days prior to study agent administration,
7. Use of any investigational agent within 3 months prior to enrollment,
8. Any condition which leads the investigator to believe that the participant cannot comply with the protocol requirements or that may place the participant at an unacceptable risk for participation.

4.3 Enrollment Procedures

Male individuals with a diagnosis of XLRS, and a parent or guardian of children <18 years of age, will be invited to give informed consent prior to screening procedures. After appropriate written informed consent/assent has been obtained, potential participants will then undergo an assessment of their medical history, physical examination, and selected clinical laboratory tests to ensure eligibility according to the protocol. Individuals who meet all inclusion and exclusion criteria will be asked to join the study.

Individuals who have been participating in the natural history study XLRS-001, entitled “Clinical Evaluation of Individuals with X-linked Retinoschisis (XLRS)”, may discontinue their participation in that study and be enrolled in the present study if all appropriate entry criteria are met. In this case, results of the evaluations from the most recent visit in the natural history study, excluding OCT, can be used as part of the appropriate screening visit evaluations for the present study, as long as all the evaluations were conducted per the current Study Manual of Procedures and Reading Center Manual of Procedures and the visit occurred no more than 3 months prior to the planned date of study agent administration. If the most recent visit occurred more than 3 months prior to the planned date of study agent administration, all screening visit examinations will be performed.

Subjects may withdraw or be withdrawn from the study for any of the following reasons:

- Voluntary withdrawal by the participant
- Non-compliance with the protocol requirements
- Investigator decision that withdrawal is in the participant’s best interest
- Termination of the study for any reason (e.g. based on a decision by the DSMC, regulatory agencies or the sponsor)

The reason for withdrawal will be documented for each subject.

Subjects who prematurely discontinue from the study before administration of rAAV2tYF-CB-hRS1 will be replaced. These subjects will have no further follow-up visits (except to follow up any AE related to participation in the study).

Subjects who prematurely discontinue from the study after administration of rAAV2tYF-CB-hRS1 will not be replaced, unless they are lost to follow-up before a DSMC review at which the safety data from their Week 2 visit is required for a decision to progress to the next dose treatment group. All subjects who prematurely discontinue from the study after administration of the study agent will be encouraged to complete an early termination (ET) visit.

5 STUDY AGENT

5.1 Description

rAAV2tYF-CB-hRS1 is a non-replicating, rep/cap-deleted, recombinant adeno-associated virus (rAAV) vector containing cDNA encoding the human retinoschisin (RS1) protein.

The vector contains AAV serotype 2 inverted terminal repeats and an expression cassette consisting of a CMV enhancer/chicken beta actin promoter, the human RS1 cDNA and an SV40 polyadenylation sequence, and is packaged in an AAV2 capsid containing tyrosine to phenylalanine (YF) mutations in three surface-exposed tyrosine residues (AAV2tYF).

The organization of the elements in the DNA molecule is depicted in Figure 2 below.

Figure 2 Organization of the rAAV2tYF-CB-hRS1 vector

ITR = inverted terminal repeat, CB = CMV enhancer/chicken beta actin promoter, RS1 = retinoschisin, pA = polyadenylation signal

rAAV2tYF-CB-hRS1 was manufactured in compliance with current Good Manufacturing Practices (cGMP) under contract to Applied Genetic Technologies Corporation (AGTC).

5.2 Study Agent Storage and Stability

The study agent is provided as a sterile frozen liquid and must be stored at -65°C or below. Stability testing indicates rAAV2tYF-CB-hRS1 is stable for up to 3 years when stored at this temperature.

5.3 Study Agent Preparation and Dosage Levels

Three dose levels of rAAV2tYF-CB-hRS1, administered by intravitreal injection, will be evaluated in this clinical protocol. The lower dose level will be 1×10^{11} vector genomes (vg) per eye (0.07 mL at 1.43×10^{12} vg/mL). The middle dose level will be 3×10^{11} vg per eye (0.07 mL at 4.3×10^{12} vg/mL). The higher dose level will be 6×10^{11} vg per eye (0.14 mL at 4.3×10^{12} vg/mL).

Research pharmacy personnel will thaw and dilute and prepare the study agent to the correct concentration and volume according to instructions provided in the study Pharmacy Manual.

The syringe(s) containing study agent will then be maintained at 2-8°C and delivered to a designated area in the administration room. Study agent administration should occur within 4 hours of thawing and preparation.

5.4 Study Agent Administration

The participant may be started on steroids prior to the study agent administration as described in the Study Manual of Procedures.

Immediately prior to study agent administration, the participant will be placed in the supine position and the participant's eye will be prepared for injection by applying topical anesthetic and/or subconjunctival anesthetic followed by povidone-iodine. A povidone-iodine solution will then be used to cleanse the eyelids. A sterile speculum will be inserted to separate the lids.

Prior to injection the povidone-iodine solution will be reapplied to the injection site. A cotton swab soaked in topical anesthetic can be used to soften the eye prior to injection. Care should be taken not to express secretions from the meibomian glands in the lids.

Sedation for the intraocular injection may be used at the discretion of the investigator to insure that the intravitreal injection can be completed in a safe manner.

The participant will be instructed to direct his or her gaze away from the site of needle entry. After the needle penetrates the sclera the needle is advanced toward the center of the globe and the fluid is gently injected into the mid- to posterior vitreous cavity. The needle is removed and a sterile cotton swab is immediately placed over the injection site to prevent reflux.

For participants receiving the high dose in a volume of 0.14 mL, it is preferable to administer a single injection as it decreases the infection risk and it is more comfortable for the patient. However; the Investigator should confirm that there is perfusion of the retina after injection and be prepared to address unusually high or prolonged increase in IOP, if needed. Performing an anterior chamber tap (paracentesis) before this single injection is at Investigator's discretion. If the dose is alternatively administered as two injections of 0.07 mL, the optic disc should be visualized after the first 0.07 mL is injected to confirm that there is perfusion of the retina, before the second 0.07 mL is injected.

After study agent administration is completed, the participant will remain in the supine position and the IOP will be measured about 30 minutes after the injection. The participant will remain under direct observation until the IOP returns to a value less than 30 mm Hg. Prolonged or unusually high IOP will be managed using standard methods at the discretion of the investigator.

The participant will be instructed to notify the investigator immediately if symptoms of eye pain, redness, photophobia or diminished vision develop.

5.5 Dose-limiting Toxicity

- Every AE recorded in this study must be assessed by the investigator to determine whether or not it meets the definition of a dose-limiting toxicity (DLT).
- A DLT is defined as any toxicity determined by the investigator to be related to the study agent and deemed serious or sufficiently severe to preclude dose escalation.
- For the purpose of this study, any grade 4 or 5 event (per the Common Terminology Criteria for Adverse Events (CTCAE) v4.0 criteria) that is assessed as possibly, probably or definitely related to the study drug will be considered to be a DLT.

5.6 Dosing Plan

Within Groups 1A, 1B, 2, 2A, and 3, enrollment of participants will be staggered by at least 2 weeks to allow adequate time for review of safety information by the investigators and sponsor.

Within group 4, enrollment of the first 3 pediatric participants in each group will be staggered by at least 2 weeks to allow adequate time for review of safety information by the investigators and sponsor.

If any participant within any study group develops a dose-limiting toxicity (as defined in Section 5.5), enrollment of additional participants will not proceed until review by the DSMC.

Enrollment will begin with Group 1A and will proceed to subsequent groups after review of safety data by the DSMC. After review of safety data from Group 1A, participants will be enrolled in Groups 1B and 2 concurrently. After review of safety data from Groups 1A, 1B and 2, participants will be enrolled in Group 3. After review of safety data from Groups 1A, 1B, 2 and 3, participants ≥18 years of age will be enrolled in Group 4. As ocular inflammation has been seen in the first 12 subjects enrolled in the study and pediatric participants might be more prone to inflammation, pediatric participants (age 6-17) in this study will first be treated at the middle dose (Group 2A) prior to the enrollment in Group 4.

Each safety review will be conducted based on data for all enrolled participants accrued up to and including the visit 2 weeks after administration of rAAV2tYF-CB-hRS1 to the last participant enrolled in Group 1A (for the first review), 2 weeks after administration of rAAV2tYF-CB-hRS1 to the last participant enrolled in Groups 1B and 2 (for the second review), 2 weeks after administration of rAAV2tYF-CB-hRS1 to the last participant enrolled in Group 3 (for the third review), or 2 weeks after administration of rAAV2tYF-CB-hRS1 to the last participant enrolled in Group 2A (for the fourth review),

The length of time between dosing participants may be extended if the investigators, sponsor, DSMC or FDA believes that additional time is needed to ensure safety of the participants. (See Section 7 for additional information on safety monitoring.)

5.7 Prior and Concomitant Therapy

To reduce the risk of intraocular inflammation, participants may be placed on prophylactic steroids prior to the study agent administration as described in the Study Manual of Procedures.

Potential participants who are taking a carbonic anhydrase inhibitor can be recruited for enrollment in this study but they will be required to discontinue this treatment at least 3 months prior to receiving study agent in order to meet the eligibility criteria.

Participants in all groups will be advised not to take any carbonic anhydrase inhibitor medication during the 6 months after study agent administration. At the Month 6 visit, the investigator may discuss potential use of carbonic anhydrase inhibitor with the subject. Short term carbonic anhydrase inhibitor use to treat IOP spikes after intravitreal study agent administration is allowed.

Participants will be instructed to discontinue aspirin, aspirin-containing products, and any other drugs that may alter coagulation, at least 7 days prior to study agent administration. Participants will be told they may resume taking these types of products starting the day after study agent administration.

Elevated intraocular pressure will be treated using standard methods at the discretion of the investigator.

5.8 Accountability Procedures for the Study Agent

Study agent will be dispatched to the site after receipt of required documents in accordance with applicable regulatory requirements and AGTC procedures.

Study agent must be administered according to procedures described herein. Only subjects enrolled in the study may receive study product, in accordance with all applicable regulatory requirements. Only authorized site personnel may administer study agent. Study agents must be stored in a secure temperature-controlled area with access limited to the investigator and authorized study site personnel.

The investigator is responsible for study agent accountability, reconciliation and record maintenance. In accordance with all applicable regulatory requirements, the investigator or designated study site personnel must maintain study agent accountability records throughout the course of the study. This will include documentation of the amount of study agent received from AGTC and the amount administered to study participants.

6 STUDY PROCEDURES/EVALUATIONS

Study agent administration will occur on Day 0. There will be a screening visit that will occur within 3 months prior to study agent administration and a baseline visit that will occur within 7 days prior to study agent administration. There will be an additional 10 visits (including Day 0) during the first year of this study and annual long-term follow-up visits during the subsequent 4 years.

Individuals who have been participating in the natural history study XLRS-001, entitled “Clinical Evaluation of Individuals with X-linked Retinoschisis (XLRS)”, may discontinue their participation in that study and be enrolled in the present study if all appropriate entry criteria are met. In this case, results of the evaluations from the most recent visit in the natural history study, excluding OCT, can be used as part of the appropriate screening visit evaluations for the present study, as long as all the evaluations were conducted per the current Study Manual of Procedures and Reading Center Manual of Procedures and the visit occurred no more than 3 months prior to the planned date of study agent administration. If the most recent visit occurred more than 3 months prior to the planned date of study agent administration, all screening visit examinations will be performed.

A Time and Events Schedule of study procedures and evaluations is provided in Appendix A. The windows for the timing of the study visits are provided in the Study Manual of Procedures.

6.1 Informed Consent/Assent

Potential participants (and a parent or guardian of children <18 years of age) will be invited to provide written informed consent (and assent from children <18 years of age) prior to any screening evaluations. An investigator or study coordinator will provide a full explanation of the study, including potential risks of participation and that they may withdraw from the study at any time and for any reason without jeopardizing their future treatment, and an investigator will provide answers to all questions raised by the potential participant (and a parent or guardian of children <18 years of age). After ensuring that all questions have been answered, written documentation of the informed consent/assent decision will be obtained.

No study-related procedures will be performed until after informed consent/assent is obtained.

A list will be maintained of all potential participants who participate in screening that will include the person’s initials, the date of screening, the outcome of screening (i.e. eligible or not eligible) and, for persons who do not enroll in the study, the reason for non-enrollment.

6.2 DNA Testing

If a potential participant has not previously had a mutation in the RS1 gene documented by genetic testing, a blood or saliva sample will be obtained for genetic testing to confirm the subject has a mutation in the RS1 gene.

6.3 Medical History and Physical Examination

A medical history will be obtained, including demographic information (date of birth, gender, race and ethnic background), at the screening visit.

A complete physical examination will be performed at the screening or baseline visit, to include vital signs (oral temperature, heart rate, respiratory rate and blood pressure), height, weight and examination of major organ systems (head/eyes/ears/nose/throat, neck, cardiovascular, respiratory, abdomen, musculoskeletal/extremities, skin, lymph nodes and neurological).

A clinical evaluation, consisting of an interim medical history, including use of carbonic anhydrase inhibitors and other medications, a review of AEs and a symptom-directed physical examination, will be obtained at all other visits.

6.4 Hematology and Clinical Chemistries

Hematology and clinical chemistry will be obtained at the screening or baseline visit and at visits 7 and 14 days after study agent administration. Hematology will include hemoglobin, hematocrit, white blood cell count and platelet count. Clinical chemistry will include sodium, potassium, chloride, total protein, albumin, calcium, phosphorous, glucose, blood urea nitrogen, creatinine, total bilirubin, alkaline phosphatase, lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase.

6.5 Ocular Fluids

Ocular fluids collected during procedures may be stored and used for future analyses.

6.6 Coagulation

Coagulation tests (prothrombin time and partial thromboplastin time) will be obtained at the screening or baseline visit.

6.7 Immunology

Serum to measure antibodies to AAV will be obtained at the screening visit and at visits 1, 3, 6 and 12 months after study agent administration. Serum to measure antibodies to RS1 will be collected at the baseline visit, at visits 1, 3 and 12 months after study agent administration.

Peripheral blood mononuclear cells (PBMC) will be collected at the baseline visit and at visits 1, 2 and 3 months after study agent administration. Aliquots of these PBMC samples will be stored for possible future analyses including measurement of T cell responses to AAV or RS1.

6.8 Vector Analysis

Whole blood for detection of vector DNA by polymerase chain reaction (PCR) assay will be obtained at the baseline visit and at visits 1, 7 and 14 days after study agent administration.

6.9 Ophthalmic Examinations

Ophthalmic examinations of both eyes will be performed at the screening and baseline visits and at each visit after study agent administration, including each long-term follow-up visit. These examinations will include slit lamp examination, tonometry, indirect ophthalmoscopy and retinal biomicroscopy. Signs of inflammatory responses will be quantified using a standard 0 to 4+ cells and flare grading system [59]. On the visit at which the study agent is administered, IOP in the study eye will be measured about 30 minutes after study agent administration is completed.

6.10 Visual Function Measurements

All visual function measurements will be obtained for both eyes at each visit when such testing is performed.

Best corrected visual acuity (BCVA) testing will be performed once at the screening visit, twice at the baseline visit, and once at each visit after study agent administration, including each long-term follow-up visit. BCVA will be determined using a standard Early Treatment of Diabetic Retinopathy Study (ETDRS) visual acuity testing protocol.

Visual field and microperimetry testing will be performed once at the screening visit, twice at the baseline visit, once at visits 1, 2, 3, 6, 9, and 12 months after study agent administration. Visual field testing will be performed using the Haag Streit Octopus 900 perimeter. Static perimetry will use the German Adaptive Thresholding Estimation (GATE) method and kinetic perimetry will use the Semi-automated Kinetic Perimetry (SKP) method. Microperimetry will be performed using the MP1 microperimeter.

Reading speed and contrast sensitivity testing will be performed once at the screening visit, twice at the baseline visit, and once at visits 3, 6, 9, and 12 months after study agent administration. Reading speed will be measured using MNREAD charts. Contrast sensitivity will be measured using Pelli-Robson charts.

Additional non-invasive measures of visual function may also be performed at the discretion of the investigator.

6.11 Electroretinography

Electroretinography testing of both eyes will be performed at the screening and baseline visits and at visits 3, 6 and 12 months after study agent administration, using both full-field (ffERG) and multifocal (mfERG) procedures.

6.12 Optical Coherence Tomography

Retinal anatomy will be evaluated by spectral domain optical coherence tomography (SD-OCT) scans of both eyes once at the screening visit, twice at the baseline visit, and once at visits 1, 2, 3, 6, 9 and 12 months after study agent administration. Additional SD-OCT scans may be obtained at the discretion of the investigator. Each SD-OCT will generate macula volume scans and high resolution horizontal and vertical scans centered on the fovea.

6.13 Fundus Photography

Fundus photography will be performed at the baseline visit and at the visit 12 months after study agent administration.

6.14 Quality of Life

For participants ≥ 16 years of age, quality of life will be assessed using the 25-Item National Eye Institute Visual Function Questionnaire (VFQ-25) [60]. The questionnaire will be administered in an interview format at the screening and baseline visits and at visits 3, 6, 9 and 12 months after study agent administration.. For participants <16 years of age, a pediatric quality of life questionnaire may be administered.

6.15 Long-term Follow-up

After the completion of 9 study visits over the course of one year after study agent administration, long-term follow-up will be conducted annually for an additional 4 years after the Month 12 visit. At each of these visits, testing will include clinical evaluation, visual acuity and ophthalmic examinations. In accordance with regulatory guidance for gene therapy clinical trials, information will also be obtained about any cancer, neurological, autoimmune or hematological disorder that developed or worsened since the last visit in the form of a long-term follow-up questionnaire. The questionnaire is provided in Appendix B.

7 ASSESSMENT OF SAFETY

7.1 Specification of Safety Parameters

7.1.1 Primary Endpoint

The primary objective of this study is to evaluate the safety of rAAV2tYF-CB-hRS1 administered by intravitreal injection in patients with XLRS. The primary safety parameter will be the frequency of Grade 3 or 4 local (ocular) or systemic AEs that occur during the 12 months after study agent administration.

7.1.2 Secondary Endpoints

Secondary safety parameters will include the following:

- The frequency of all AEs, including serious AEs,
- The frequency of abnormal hematology or clinical chemistry parameters,
- Changes in titers of serum antibodies or IFN-γ ELISPOT responses to RS1.

7.2 Assessing, Recording, and Analyzing Safety Parameters

Local (ocular) and systemic AEs will be assessed using CTCAE v4.0 criteria and recorded at every visit. Ophthalmic examinations, as described in Section 6.9, will be performed before and after study agent administration. Slit lamp biomicroscopy of anterior and posterior segment, tonometry and indirect ophthalmoscopy will be the principal method to detect ocular AEs.

Non-ocular AEs will be assessed by performing a medical history and physical examination before study agent administration, obtaining an interim medical history and symptom-directed physical exam at each visit after study agent administration, and performing tests of hematology and clinical chemistry before and after study agent administration as described in Section 6. Non-ocular AEs will be assessed by performing a medical history and physical examination before study agent administration, obtaining an interim history and symptom-directed physical exam at each visit after study agent administration, and performing tests of hematology and clinical chemistry parameters before and after study agent administration as described in Section 6.

Enrolled participants will have frequent follow-up visits during the first year after study agent administration. To monitor for delayed AEs and assess the duration of any visual changes that occur, long-term follow-up will be conducted annually for 4 years after completion of the Month 12 visit after the study drug administration. In accordance with regulatory guidelines for gene therapy clinical trials, at these visits, information will be obtained about any cancer, neurological, autoimmune or hematological disorder that developed or worsened since the last visit.

7.2.1 Adverse Events and Serious Adverse Events

7.2.1.1 Definition of an Adverse Event

The International Conference on Harmonisation (ICH) E6 Good Clinical Practice (GCP) guidelines define an AE as “any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.” An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews or by a study recipient presenting for medical care. All AEs must be graded for intensity and relationship to study product.

An AE **does** include:

- Exacerbation of a pre-existing illness,
- An increase in frequency or intensity of a pre-existing episodic event or condition,
- A condition detected or diagnosed after study agent administration even though it may have been present prior to the start of the study,
- Continuous persistent disease or symptoms present at baseline that worsen following the start of the study.

An AE **does not** include:

- A medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure may be an AE,
- A pre-existing disease or conditions present or detected at the start of the study that does not worsen,
- Situations where an untoward medical occurrence has not occurred (e.g., normal pregnancy, hospitalizations for cosmetic elective surgery, social and/or convenience admissions),
- Overdose of either study agent or concurrent medication without any signs or symptoms.

AEs may include pre- or post-treatment events that occur as a result of protocol-mandated procedures (e.g., invasive procedures, modification of subject’s previous therapeutic regimen). Intercurrent illnesses that occur after screening but before starting treatment, and that are not the result of protocol-mandated procedures, do not need to be captured as AEs.

7.2.1.2 Intensity of an Adverse Event

All AEs will be assessed by the investigator using the following guidelines to quantify intensity:

- Mild: Events that do not interfere with daily activities and require no medical intervention.
- Moderate: Events that cause some interference with daily activities and may require simple medical interventions.
- Severe: Events that prevent usual daily activities and require medical intervention.
- Life-threatening: Any adverse drug experience that places the subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.
- Fatal: Any AE that causes the death of the subject. Except for sudden and unexpected death, death is not an AE, but rather the event that caused the subject to die is the AE.

Changes in the intensity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

7.2.1.3 Relationship to Study Agent

The investigator's assessment of an AE's relationship to study agent is part of the documentation process, but it is not a factor in determining what is or is not reported as an AE in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study agent assessed using the following terms: associated or not associated. "Associated with the use of the study agent" means that there is a reasonable possibility that the experience may have been caused by the study agent [61]. In a clinical trial the study agent must always be suspect. To help in assessing the relationship to study agent, the following guidelines are used.

- **Associated** – The event is temporally related to the administration of the study agent and no other etiology explains the event.
- **Not Associated** – The event is temporally independent of study agent and/or the event appears to be explained by another etiology.

Events assessed as being associated with study agent will be further classified as possibly, probably or definitely related to study agent.

Events assessed as being not associated with study agent will be further classified as probably not or definitely not related to study agent.

7.2.1.4 Relationship to Intravitreal Injection Procedure

All AEs will also be assessed for their relationship to the intravitreal injection procedure used to administer the study agent, using the same categories as described in Section 7.2.1.3.

7.2.1.5 Serious Adverse Event (SAE)

An SAE is any untoward medical occurrence that at any dose:

- Results in death,
- Is life-threatening. Any AE that places the subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred. This **does not** include a reaction that, had it occurred in a more serious form, might have caused death.
- Requires in-patient hospitalization or prolongation of existing hospitalization. This **does not** include hospitalization for elective or pre-planned surgery, *unless* prolonged hospitalization occurs due to complications from the surgical procedure,
- Results in persistent or significant disability or incapacity,
- Results in a congenital anomaly/birth defect,
- Is an event that required intervention to prevent permanent impairment or damage,
- Any other important medical events that do not result in death, are not life-threatening, or do not require hospitalization may be considered SAEs when, based upon appropriate medical judgment, the event may jeopardize the participant and might require medical or surgical intervention to prevent one of the previously described outcomes. Examples of such medical events include allergic bronchospasm requiring invasive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

7.3 Reporting Procedures

7.3.1 Adverse Events

All AEs should be captured on the appropriate electronic case report form (eCRF). Information to be collected includes event description, date of onset, intensity, relationship to study agent, relationship to the intravitreal injection procedure used to administer the study agent, action taken, date of resolution or stabilization of the event, seriousness, and outcome (resolved, ongoing or death caused by the AE). All AEs occurring while on study must be documented and treated appropriately regardless of relationship. All AEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or the subject is lost to follow-up.

Any medical condition that is present at the time that the subject is screened should be considered as a pre-existing condition and not reported as an AE; however, if it deteriorates at any time during the study it should be recorded as an AE.

7.3.2 Serious Adverse Events

All AEs that meet the criteria for an SAE as defined in Section 7.2.1.5 must be reviewed and evaluated by a study physician and captured on the appropriate SAE eCRF. The SAE eCRF must be complete within the following timelines:

- Every AE with onset during or after study agent administration that meets the protocol defined criterion for a designation of serious or a DLT must be reported to the Sponsor by recording on the subject's AE eCRF within 24 hours of the Investigator or study staff's first knowledge of the event.
- Entry of SAE or DLT information in the eCRF will trigger an e-mail to the Sponsor to take appropriate action in response to the information.
- In any instance in which the database cannot be accessed, a paper SAE form must be completed and faxed to the Sponsor (at AGTC fax 386 462 7396), followed by a phone call within 24 hours of the Investigator or study staff's first knowledge of the event.

A separate set of SAE eCRF pages should be used for each SAE. However, if at the time of initial reporting, multiple SAEs are present that are temporally and/or clinically related, they may be reported on the same SAE eCRF page.

DLT reporting will follow the same timeline as SAE reporting.

The investigator should attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE and/or SAE and not the individual signs/symptoms. New or updated information should be recorded on the originally completed SAE eCRF pages.

If a subject dies during participation in the study, a copy of any post-mortem findings, including histopathology, should be provided when available as an attachment to an updated SAE eCRF page.

Other supporting documentation of the event may be requested and should be provided as soon as possible.

Questions about SAE reporting can be referred to Theresa Heah, MD at AGTC telephone 617-843-5728, extension 7241.

7.3.3 Regulatory Reporting

Following notification from the investigator, the sponsor will report events that are both serious and unexpected and that are associated with study agent to the US Food and Drug Administration (FDA) within the required timelines as specified in 21 CFR 312.32, *i.e.*, fatal and life-threatening events within 7 calendar days (by telephone or fax) and all other SAEs in writing within 15 calendar days. These SAEs will also be reported to the Institutional Review Board (IRB) at each site, the NIH Office of Biotechnology Activities, and other site-specific committees according to regulatory requirements. SAEs designated as not associated with study product will be reported in a summary format to the DSMC during each between-cohort review and to the FDA at least annually.

7.3.4 Reporting of Pregnancy

Not applicable - females will not participate in this study.

7.3.5 Procedures to be Followed in the Event of Abnormal Laboratory Test Values

Any clinically significant abnormal laboratory test result will be recorded as an AE, and if it meets the criteria defined in Section 7.2.1.5 it will be reported as an SAE.

7.3.6 Follow-up of Adverse Events

All SAEs and DLTs must be followed and treated as appropriate until resolution. An SAE or DLT is considered resolved when the subject returns to their baseline condition, or when the event has stabilized, as in the case of an event with sequelae, in which case the event will be noted as “resolved with sequelae”.

If an SAE or DLT is still ongoing at the visit 12 months after study drug administration, follow-up of the event will be done at each follow-up visit until the end of the study.

If an SAE or DLT is still ongoing when the subject is lost to follow-up, the study site staff needs to complete the following steps to ensure the proper follow up the SAE or DLT:

- Complete and document two separate attempts to contact the subject and/or their emergency contact by telephone at an appropriate clinically indicated interval for follow-up of the event;
- If attempts to contact the subject are unsuccessful, send a certified letter to the subject's last known address requesting that the subject contact the study site.

Non-serious AEs that are assessed as “related” to the study agent will be followed and treated as appropriate until the end of the study, including evaluation at long-term follow-up visits if the event is ongoing at the visit 12 months after the study drug administration.
Non-serious AEs that are evaluated as “not related” to the study agent, and that are ongoing at the visit 12 months after the study drug administration, will be characterized as “ongoing” at that visit, and further follow up will not be required.

The investigator is responsible for ensuring that follow-up includes any supplemental investigations that may be indicated to elucidate as completely as practical the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

7.4 Data and Safety Monitoring Plan

7.4.1 Temporary Suspension of Study Agent Administration

Within groups 1A, 1B, 2, 2A, and 3, enrollment of participants will be staggered by at least 2 weeks to allow adequate time for review of safety information by the investigators and sponsor. Within group 4, enrollment of the first 3 pediatric participants in each group will be staggered by at least 2 weeks to allow adequate time for review of safety information by the investigators and sponsor. If any participant within any study group develops a dose-limiting toxicity (as defined in Section 5.5), enrollment of additional participants will not proceed until review by the DSMC.

7.4.2 Criteria for Enrollment in Subsequent Groups

Enrollment will begin with Group 1A and will proceed to subsequent groups only after review of safety data by the DSMC. After review of safety data from Group 1A, participants will be enrolled in Groups 1B and 2 concurrently. After review of safety data from Groups 1A, 1B and 2, participants will be enrolled in Group 3. After review of safety data from Groups 1A, 1B, 2 and 3, participants ≥18 years of age will be enrolled in Group 4 As ocular inflammation has been seen in the first 12 subjects enrolled in the study and pediatric participants might be more prone to inflammation, pediatric participants (age 6-17) in this study will first be treated at the middle dose (Group 2A) prior to the enrollment at Group 4.

Each safety review will be conducted based on data for all enrolled participants accrued up to and including the visit 2 weeks after administration of rAAV2tYF-CB-hRS1 to the last participant enrolled in Group 1A (for the first review), 2 weeks after administration of rAAV2tYF-CB-hRS1 to the last participant enrolled in Groups 1B and 2 (for the second review) ,2 weeks after administration of rAAV2tYF-CB-hRS1 to the last participant enrolled in Group 3 (for the third review), or 2 weeks after administration of rAAV2tYF-CB-hRS1 to the last participant enrolled in Group 2A (for the fourth review) .

The length of time between dosing participants may be extended if the investigators, sponsor, DSMC or FDA believes that additional time is needed to ensure safety of the participants.

8 ASSESSMENT OF EFFICACY

The secondary objective of this study is to evaluate the efficacy of rAAV2tYF-CB-hRS1 in patients with XLRS. The efficacy parameters in this study will be:

8.1 Specification of Efficacy Parameters

- Changes in best corrected visual acuity,
- Changes in the size of schisis cavities on OCT scans,
- Changes in ERG responses,
- Changes in static and kinetic visual fields,
- Changes in microperimetry,
- Changes in reading speed,
- Changes in contrast sensitivity,
- Changes in ffERG,
- Changes in mfERG,
- Changes in quality of life questionnaire responses.

8.2 Assessing, Recording, and Analyzing Efficacy Parameters

Efficacy parameters will be assessed as described in Sections 6.10, 6.10, 6.12 and 6.14.

9 CLINICAL MONITORING STRUCTURE

To ensure that the study is conducted in compliance with AGTC, FDA, and ICH guidelines, the clinical trial will be monitored by AGTC or a CRO representing AGTC.

9.1 Site Monitoring Plan

A Clinical Research Associate (CRA) will be assigned to monitor the investigational site and will be given full authorization to contact and visit the investigational site as required to verify adherence to the protocol and any amendments, the completeness, accuracy and consistency of the data, adherence to ICH GCP guidelines, and to ensure that all applicable regulatory requirements are being met.

An initiation meeting will be conducted at which the protocol and the data collection procedures will be reviewed with the relevant members of the site investigational team. Throughout the course of the study the CRA will maintain regular contact with the site investigational team and will visit the site at regular intervals appropriate to the subject recruitment rate and the volume and quality of the data. Monitoring visits to the site will be arranged in advance with the relevant members of the site investigational team.

In particular, the CRA will undertake the following activities on a regular basis at the site, as appropriate to the study and recruitment status at the site:

- Review of subject recruitment,
- Review of eCRFs,
- Verification of study data against source data,
- Collection of study data,
- Ensure appropriate reporting and follow-up of SAEs,
- Review of site files and study-related documentation,
- Inspection of storage, stock levels and accountability for all study materials,
- Ensure investigational team and facilities remain suitable and adequate for the conduct of the study,
- Identification and resolution of any study-related queries or problems.

It is understood that the CRA will have full authorization to inspect all records relating to the trial on request, provided that subject confidentiality is maintained and the inspection is conducted in accordance with applicable local regulations. It is also understood that the investigator will confirm his agreement, prior to the study commencement, to co-operate with the CRA in ensuring that any problems detected during the course of the monitoring visits are adequately addressed in a timely manner.

At the end of the study, the CRA will work with the investigator to ensure the return of all study data to the sponsor. The CRA will ensure that data clarifications are completed, accounting and reconciliation of used and unused study agent is performed, the used and unused study agent is disposed of as requested by the sponsor, and the site study records are reviewed for completeness.

10 STATISTICAL CONSIDERATIONS

10.1 Overview and Study Objectives

This is a non-randomized, open-label study dose escalation study to evaluate the safety and efficacy of rAAV2tYF-CB-hRS1 in patients with XLRS.

10.2 Study Population

Approximately 27 male individuals with XLRS will be enrolled in this study.

10.3 Study Design

Each participant will receive rAAV2tYF-CB-hRS1 by intravitreal injection in one eye on a single occasion as outlined in the schematic below.

Schematic of Study Design:

Group [a]	Age (yr)	Number of subjects	Dose level		
			vg/mL	volume	vg per eye
1A	≥ 18	3	1.43×10^{12}	0.07 mL	1×10^{11}
1B	≥ 18	3	1.43×10^{12}	0.07 mL	1×10^{11}
2	≥ 18	3	4.3×10^{12}	0.07 mL	3×10^{11}
2A	6-17	Up to 6	4.3×10^{12}	0.07 mL	3×10^{11}
3	≥ 18	3	4.3×10^{12}	0.14 mL	6×10^{11}
4	≥ 6	Up to 15	MTD [b]	MTD	MTD

[a] Visual acuity not better than 58 ETDRS letter score in Group 1A, 63 in Groups 1B, 2, 2A, & 3, 68 in Group 4.

[b] MTD = maximum tolerated dose determined in Groups 1A, 1B, 2 and 3 for adults; in Groups 1A, 1B, 2, and 3 (adult participants only) and 2A (pediatric participants only) for pediatric participants.

Participants in Groups 1A, 1B, 2 and 3 will be at least 18 years of age and receive the vector at a lower dose (Group 1), middle dose (Group 2) or higher dose (Group 3). Participants in Group 2A will be 6-17 years of age and receive the vector at the middle dose level. Participants in Group 4 will be at least 6 years of age and receive the vector at the maximum tolerated dose (MTD) determined in Groups 1A, 1B, 2 and 3 for adults; in Groups 1A, 1B, 2, and 3 (adult participants only) and 2A (pediatric participants only) for pediatric participants.

Participants in all groups will be selected from individuals who are not currently being treated with a carbonic anhydrase inhibitor and have not been treated with any carbonic anhydrase inhibitor within the 3 months prior to Day 0 (study agent administration).

10.4 Study Hypotheses

The null hypothesis for safety is that there will be no dose-limiting toxicity. The null hypothesis for efficacy endpoints is that there will be no biological activity at the dosage levels tested.

10.5 Sample Size Considerations

A sample size of 3 participants per group is typical of the dose escalation component in Phase 1/2 clinical trials of gene therapy products, especially for diseases with low prevalence. This small sample size will obviously limit the power of the study, so that only very large treatment effects can be detected. For example, if no dose-limiting toxicity is detected among the first 9 participants, the 95% confidence interval for the rate of dose-limiting toxicity would be from 0 to 33.63%, and if no dose-limiting toxicity is detected among the maximum of 27 participants in the study, the 95% confidence interval for the rate of dose-limiting toxicity would be from 0 to 12.77%,.

10.6 Participant Enrollment and Follow-Up

Enrollment will begin with Group 1A and will proceed to subsequent groups after review of safety data by the DSMC. After review of safety data from Group 1A, participants will be enrolled in Groups 1B and 2 concurrently. After review of safety data from Groups 1A, 1B and 2, participants will be enrolled in Group 3. After review of safety data from Groups 1A, 1B, 2 and 3, participants ≥18 years of age will be enrolled in Group 4 As ocular inflammation has been seen in the first 12 subjects enrolled in the study and pediatric participants might be more prone to inflammation, pediatric participants (age 6-17) in this study will first be treated at the middle dose (Group 2A) prior to the enrollment at Group 4.

Enrolled participants will have frequent follow-up visits during the first year after study agent administration. To monitor for delayed AEs and assess the duration of any visual changes that occur, participants will be followed annually for an additional 4 years after the Month 12 visit.

10.7 Analysis Plan

Due to the small sample size within each treatment group, no inferential comparisons among groups are planned.

When the use of descriptive statistics to assess group characteristics or differences is required, the following methods will be used: for categorical variables, the number and percent in each

category; for continuous variables, the mean, median, standard deviation, quartiles, and range (min, max).

10.7.1 Baseline Characteristics

Listings and descriptive statistics will be provided for baseline characteristics including demographics (race, ethnicity, age, sex, height and weight), clinical variables (temperature, blood pressure, pulse and respiratory rate) and laboratory measurements (hematology and clinical chemistry parameters).

10.7.2 Safety Analysis

Safety will be monitored by evaluation of ocular and non-ocular AEs and hematology and clinical chemistry parameters.

Adverse experiences will be coded into MedDRA preferred terms. The number and percentage of participants within each treatment assignment experiencing each specific adverse experience will be tabulated by intensity and by relationship to treatment. For the calculations in these tables, each participant's adverse experience will be counted once under the maximum intensity or the strongest recorded causal relationship to treatment.

A complete listing of serious adverse experiences for each participant will provide details including intensity, relationship to treatment, onset, duration and outcome.

Listings and descriptive statistics will be provided for each time point for all laboratory parameters.

10.7.3 Efficacy Analysis

Efficacy will be measured by evaluation of visual acuity, schisis cavity volume on OCT scans, static and kinetic visual fields measured using the Octopus perimeter, microperimetry, reading speed, contrast sensitivity, ERG, and quality of life questionnaires.

Visual acuity data will be presented as the ETDRS letter score at each time point.

Volumetric analysis of schisis cavity size will be quantified using a method developed at the Casey Eye Institute.

For visual fields, mean sensitivity, mean defect, square root of the loss variance, volume of full field hill of vision, 30 degree hill of vision, total volume loss, the area of kinetic perimetry isopters in mm^2, and the mean luminance sensitivity for the entire test field in decibel steradians, will be recorded for each eye and at each time point.

For microperimetry, the equivalent of the 10-2 stimulus pattern will be used with a Nidek MP-1, microperimeter. The initial analysis will evaluate total sensitivity over time. Subsequent analyses will evaluate focal regions of change or point-by-point regression over time.

For reading speed, the reading acuity, critical print size and maximum reading speed will be recorded.

For contrast sensitivity, the number of letters read will be recorded.

The ffERGs responses will be analyzed by recording amplitude and implicit time measurements for each eye for standard ISCEV responses. Additional analyses to isolate the rod-only b-wave response may also be performed.

The mfERG responses will be analyzed by recording average latency and amplitudes for each of 6 standard rings for a 103 hexagon display.

Quality of life questionnaires will be scored using the NEI VFQ-25 scoring algorithm.

10.7.4 Other Analyses

Immune responses will be measured quantitatively (antibody titer, spot-forming cells per 10^6 PBMC) and also as binary indicators of whether there is a measurable response to the target protein.

Within each treatment group, immunogenicity analyses will be performed by using descriptive statistics and by tabulating the frequency of a positive response for each assay and for each time point that an assessment is performed. Immune responses will be analyzed as time point-specific and cumulative prevalence rates.

10.7.5 Interim Analyses

Enrollment will begin with Group 1A and will proceed to subsequent groups after review of safety data by the DSMC. After review of safety data from Group 1A, participants will be enrolled in Groups 1B and 2 concurrently. After review of safety data from Groups 1A, 1B and 2, participants will be enrolled in Group 3. After review of safety data from Groups 1A, 1B, 2 and 3, participants ≥18 years of age will be enrolled in Group 4. After review of safety data in Group 2A, the pediatric participants 6-17 years of age may be enrolled in Group 4.

Before each DSMC review, the investigators and sponsor will prepare a summary of all available data. For DSMC reviews related to enrollment in subsequent groups, this summary will include data at least through the visit 2 weeks after study agent administration for all available participants.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The study site will maintain appropriate medical and research records for this trial, in compliance with ICH E6 GCP Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. The site will permit authorized representatives of the sponsor and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress.

Source data are all information, original records and certified copies of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, X-rays, and subject files and records kept at the pharmacy, at the laboratories, and medicotechnical departments involved in the clinical trial.

12 QUALITY CONTROL AND QUALITY ASSURANCE

Following written standard operating procedures, study monitors will verify that the clinical trial is conducted and data are generated, recorded and reported in compliance with the protocol, GCP, and applicable regulatory requirements. Reports on monitoring activities will be submitted to the sponsor.

The sponsor will secure agreement from all involved parties to ensure direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

The sponsor will implement quality control procedures to ensure the accuracy of the study database. Any missing data or data anomalies will be communicated to the site for clarification/resolution.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 Declaration of Helsinki

The investigator will ensure that this study is conducted in conformity with the current revision of the Declaration of Helsinki, or with ICH GCP regulations and guidelines, whichever affords the greater protection to the subject.

13.2 Institutional Review Board

The study site will provide for the review and approval by an appropriate IRB of this protocol, the associated informed consent documents, and other materials that may be provided to potential study participants. Any amendments to the protocol, consent documents or associated materials must also be approved before they are placed into use.

13.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study, including screening procedures, and continuing throughout the individual's study participation. Appropriate discussion of risks and possible benefits of this study will be provided to the subjects. Consent forms describing in detail the treatment procedure and risks will be given to the subject and written documentation of informed consent is required prior to starting treatment. Consent forms will be approved by the IRB and the subject will be asked to review a written or recorded version of the document. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. Each subject will sign the informed consent document prior to any procedures being done specifically for the study. The subjects should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects may withdraw consent without penalty at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

A sample consent form for subject participation will be provided as a separate document to accompany the protocol.

13.4 Exclusion of Women, Minorities, and Children

XLRS is a recessive, X-linked condition that occurs almost exclusively in males. Although it is possible that a woman could develop XLRS by inheriting an RS1 mutation from a father with the disease and a mother who is a carrier, such individuals are exceedingly rare. Therefore this study will enroll only males.

Minorities will not be excluded from this clinical trial.

Children at least 6 years of age will be eligible to enroll in this clinical trial.

13.5 Subject Confidentiality

Research records and biological samples will be confidential to the extent permitted by law. Participants will be identified by a code, personal information from their records will not be released without the participant's prior written permission, and they will not be personally identified in any publication about this study. However, records may be reviewed by the sponsor and/or regulatory agencies.

13.6 Study Discontinuation

Subjects will not have access to study agent after completion of this study.

13.7 Storage of Samples for Future Research

Samples of serum and PBMC will be stored and may be used only for future evaluation of immune responses to AAV or RS1. Ocular fluids collected during procedures may be stored and used for future analyses.

13.8 Conflict of Interest

None of the investigators in this study has a financial conflict of interest.

14 DATA HANDLING AND RECORD KEEPING

Data must be collected in an accurate, consistent, complete and reliable manner in accordance with ICH GCP guidelines. Detailed instructions for completing forms, data handling procedures, and procedures for data monitoring will be provided in the Study Procedures Manual and the Manual of Procedures for Uploading Data to the Reading Center.

14.1 Data Management Responsibilities

The sponsor will have responsibility for data management.

14.2 Data Capture Methods

Clinical data will be captured using an eCRF that will be completed for each subject by the investigator or designee. Data should be entered on an ongoing basis and entry should be completed within 5 business days of each study visit. Investigators will be asked to edit eCRF data for completeness and consistency where necessary.

Data from the eCRF and data from central laboratories will be entered into a computer database and further quality assurance checks will be made to produce a final database for analysis.

At the end of the study the investigator must sign and date a declaration attesting to his/her responsibility for the quality of all data recorded and that the data represents a complete and accurate record of each subject's participation in the study.

14.3 Types of Data

This study will collect safety and efficacy data as described elsewhere in this protocol.

14.4 Timing/Reports

Safety data will be evaluated during DSMC reviews as described elsewhere in this protocol. All safety and efficacy data will be reviewed at the end of the study.

14.5 Study Records Retention

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party, without prior written approval of the sponsor.

The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records. The investigator will maintain all records pertaining to this study for a period of 2 years following the date a marketing application is approved for the study agent for the indication for which it is being investigated; or if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified.

14.6 Protocol Deviations

Any protocol deviations will be documented, recorded on the appropriate eCRF pages, and reported to the sponsor.

15 PUBLICATION POLICY

All data generated by this study are the property of AGTC and shall be held in strict confidence along with information furnished by AGTC. Independent analyses and/or publication of these data by the Investigator or any member of his/her staff is not permitted without prior written authorization of AGTC.

Any formal presentation or publication of data from this trial will be considered as joint publication of the Investigator(s) and appropriate sponsor personnel. For multicenter studies, it is mandatory that the first publication is based on data from all centers, analyzed as stipulated in the protocol and not by individual investigators. Investigators participating in multicenter trials agree not to present data gathered from one center or a small group of centers before the full publication, unless formally agreed to in writing. Written permission to the Investigator will be contingent on the review by the sponsor of the methodology and statistical analysis and any publications or presentation will provide for nondisclosure by AGTC's confidential or proprietary information. In all cases the parties agree to submit all manuscripts or abstracts to all other parties at least 30 days prior to submission. This will enable all parties to protect proprietary information and to provide comments based on information that may not yet be available to other parties. Authorship of the results of this study will be designated by the sponsor.

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17 SUPPLEMENTS AND APPENDICES

The following appendices will be provided with this protocol:

- Time and Events Schedule
- Long-term Follow-up Questionnaire
- Summary of Protocol Changes
- Site-specific Signature Page
- Sponsor Signature Page

The following documents will also accompany this protocol:

- Sample Informed Consent Document
- Sample Child Assent Document
- Clinical Investigator's Brochure
- Study Manual of Procedures
- Manual of Procedures for Uploading Data to the Reading Center
- Pharmacy Manual
- Conflict of Interest Statement
- Common Terminology Criteria for Adverse Events v4.0
- Study Site and Sponsor Contact List

Appendix A. Time and Events Schedule

Time relative to study agent administration	Screen[a]	BL[b]	Day				Month						Year	ET[d]
			0	1	7	14	1	2	3	6	9	12	2-5[c]	
Visit Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13-16**	
Informed consent/assent	✓													
Inclusion/exclusion criteria	✓	✓												
Initial medical history	✓													
Blood for DNA testing[e]	✓													
Physical exam[f]	✓													
Safety labs[f,g]	✓				✓	✓								✓*
Coagulation (PT and PTT)[f]	✓													
Blood for AAV antibodies	✓						✓		✓	✓		✓		✓
Blood for RS1 antibodies		✓					✓		✓			✓		✓
Blood for PBMC		✓					✓	✓	✓					✓*
Blood for vector DNA		✓		✓	✓	✓								✓*
Prophylaxis for inflammation		✓												
Study Agent Administration			✓											
Clinical Evaluation[h]		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Adverse Event Review		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ophthalmic Examinations[i]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Visual Acuity	✓	×2		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Octopus visual fields	✓	×2					✓	✓	✓	✓	✓	✓		✓
Microperimetry	✓	×2					✓	✓	✓	✓	✓	✓		✓
Reading speed	✓	×2							✓	✓	✓	✓		✓
Contrast Sensitivity	✓	×2							✓	✓	✓	✓		✓
Electroretinography[j]	✓	✓							✓	✓		✓		✓
Optical Coherence Tomography	✓	×2					✓	✓	✓	✓	✓	✓		✓
Fundus Photography		✓										✓		✓
Quality of life questionnaire	✓	✓							✓	✓	✓	✓		✓
Long-term follow-up questionnaire													✓	

[a] The screening visit should occur within 3 months before study agent administration.
[b] The baseline (BL) visit should occur within 7 days before study agent administration.
[c] Long-term follow-up (LTFU) evaluations once a year for years 2-5.
[d] An early termination (ET) visit will be sought for any subject who withdraws from the study before the Year 1 visit. Perform the evaluations indicated as ✓* if the ET visit occurs before the last visit at which these tests are required.
[e] DNA testing for participants who have not had a mutation in the *RS1* gene documented previously.
[f] Physical exam, safety labs and coagulation may be obtained at either the screening or baseline exam.
[g] Safety labs include hematology and clinical chemistry as specified in Section 6.4.
[h] Clinical evaluation consists of an interim medical history, including use of carbonic anhydrase inhibitors and other medications, review of AEs, and a symptom-directed physical examination.
[i] Ophthalmic examinations include slit lamp examination, tonometry, indirect ophthalmoscopy and retinal biomicroscopy in both eyes will be evaluated at each visit, except on Day 0 (Visit 3) when the only required exam is tonometry of the study eye about 30 minutes after study agent administration is completed.

Appendix B. Long-term Follow-up Questionnaire

LONG-TERM FOLLOW-UP QUESTIONNAIRE		**Date:**_____
Applied Genetic Technologies Corporation	Protocol Number AGTC-RS1-001	Subject ID (three digit site, four digit number) ___ ___ ___ - ___ ___ ___ ___

Adverse Events

1. Since your last evaluation for this study, have you had any unexpected illness or admission to a hospital?

   ❒ No ❒ Yes

   If yes, indicate the number of such events below, and describe each event on a Long-Term Adverse Event Report page.

   ❒ 1 ❒ 2 ❒ 3 ❒ 4 ❒ 5 ❒ 6 ❒ _____*

   * If more than 6 events, write the number of events on this line.

Malignancies

2. Since your last evaluation for this study, have you developed any new malignant tumor (have you been told that you have cancer)?

   ❒ No ❒ Yes

Neurological Disorders

3. Since your last evaluation for this study, have you developed any new disease of your nerves or nervous system, or had any worsening of an existing disease of your nerves or nervous system?

   ❒ No ❒ Yes

Rheumatologic or Other Autoimmune Disorders

4. Since your last evaluation for this study, have you developed any new rheumatologic or other autoimmune disorder, or had any worsening of an existing rheumatologic or other autoimmune disorder? (Rheumatologic disorders are those that affect joints, and autoimmune disorders are those in which your immune system attacks parts of your own body.)

   ❒ No ❒ Yes

Blood Disorders

5. Since your last evaluation for this study, have you developed any new blood disorder, or had any worsening of an existing blood disorder?

   ❒ No ❒ Yes

Appendix C. Summary of Protocol Changes

Date	Protocol Version	Summary of Changes
06 Mar 2015	1.0	Original protocol
15 Mar 2015	1.01	Added IND number Corrected temperature at which study agent is stored
25 Mar 2015	1.1	Within groups 4 and 5, enrollment of the first 3 pediatric participants in each group will be staggered by at least 2 weeks to allow adequate time for review of safety information by the investigators and sponsor. Additional information about carbonic anhydrase inhibitor use added in sections 2.3.1 and 6.7.
06 May 2015	1.2	Modified injection volumes based on vector concentration of 4.3×10^{12} vg/mL.
19 May 2015	2.0	Removed investigator signature pages and provided a site-specific signature page as an appendix. Removed section 1 and added a Sponsor and Study Site Contact List to section 17.
20 Oct 2015	3.0	Added steroid treatment for inflammation prophylaxis as recommended by the DSMC. Modified the study design to combine Groups 4 and 5 into a single group (a new Group 4), with no subject receiving a carbonic anhydrase inhibitor during the 6 months after study agent administration. Changed the point of contact for questions on SAEs from Jeffrey Chulay to Rabia Ozden. Modified the DLT definition to make the language more clear, and to leave the decision of the DLT assessment to investigator discretion. Modified the AE reporting and follow up responsibilities to make the language more clear. Corrected typographical and grammatical errors.

Date	Protocol Version	Summary of Changes
19 Apr 2016	4.0	Added risks for anterior chamber tap in Section 1.3.1. Clarified VA criteria for Groups 1A-4 by making the ETDRS letter score as primary enrollment criterion and revised ETDRS letter scores for each Group to include all ETDRS letter scores applicable to that Snellen equivalent calculated using ETDRS charts or EVA in Section 4.1. Based on discussions with investigators, revised intravitreal injection procedure language in Section 4.5 to indicate a single 0.14 mL injection is preferred. Updated static visual field analysis plan in Section 10.7.3.
17 June 2016	5.0	Revised estimated study enrollment duration pages 8 and 24. Corrected footnotes in study design table pages 9, 23 and 45. Added topical carbonic anhydrase inhibitor as an allowed medication to treat elevated IOP in section 5.7. Fixed minor typographical and formatting errors.
14 July 2017	6.0	Revised estimated study enrollment duration in the Protocol Summary and Section 3. Revised estimated number of participants in the Protocol Summary and Sections 3, 4, and 10.2. Modified the study design to include Group 2A throughout the protocol. Participants in this group will be 6-17 years old and receive the study agent at the middle dose. Added clarifications for the MTD throughout the protocol. Updated section 1.3.1 to further clarify risks related to the use of steroids. Added risks associated with anesthesia to section 1.3.1. Revised Sections 4.1 and 10.3 to include the new Group 2A and to clarify the washout period for CAIs. Updated Exclusion Criteria # 3 (section 4.3). Added clarification to Section 7.2.1.5 regarding SAE definition. Fixed minor typographical and formatting errors.

Date	Protocol Version	Summary of Changes
12 January 2018	7.0	Added risks associated with the study in Section 1.3.1. Added “Ocular Fluids” Section 6.5. Clarification added to the Storage of Samples for Future Research in Section 13.7.
21 October 2019	8.0	Removal of efficacy testing at LTFU visits: 12 month analysis revealed that AGTC-301 is generally safe and well-tolerated, but has no evidence of a treatment effect. Addition of LTFU questionnaire in Appendix B Updated publication policy

SITE-SPECIFIC APPENDIX 1

Signature Page

The following signature constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable United States federal regulations and ICH guidelines.

_____ _____/_____/_____

Investigator's Signature Date

_____

Investigator's Name

Sponsor Signature Page

The following signature constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable United States federal regulations and ICH guidelines.

Chief Medical Officer's Signature

23, OCT, 2019

Date

THERESA HEALY

Chief Medical Officer's Name