| | |
|---|---|
| Protocol Number: | AAV2-hCHM-101 |
| Protocol Title: | A Phase 1/2 Safety Study in Subjects with CHM (Choroideremia) Gene Mutations Using an Adeno-Associated Virus Serotype 2 Vector to Deliver the Normal Human CHM Gene [AAV2-hCHM] to the Retina |
| Investigational Product Name: | AAV2-hCHM |
| FDA IND Number: | 16132 |
| Sponsor: | Spark Therapeutics, Inc.<br>3737 Market Street, Suite 1300<br>Philadelphia, PA 19104<br>Telephone: [redacted]<br>Email: [redacted] |
| Protocol Date: | 08 Apr 2020 Version 10 |
| Revision History: | 04 January 2019 (Version 9)<br>27 April 2017 (Version 8)<br>15 December 2016 (Version 7)<br>29 September 2016 (Version 6)<br>13 October 2015 (Version 5)<br>13 November 2014 (Version 4)<br>17 September 2014 (Version 3)<br>13 August 2014 (Version 2)<br>5 May 2014 (Version 1)<br>3 October 2013 (Version 0) |

This study will be conducted in accordance with the standards of Good Clinical Practice (as defined by the International Conference on Harmonization), the ethical principles that have their origin in the Declaration of Helsinki and all applicable national and local regulations.

## SUMMARY OF CHANGES FROM PREVIOUS VERSION:

| SECTION: | SUMMARY OF REVISIONS MADE: | RATIONALE: |
|---|---|---|
| Table of Clinical Assessments | Years 6-15 of long-term follow up were removed from table, and the long term follow-up period concludes after year 5 visit. | Changes made per January 2020 FDA guidance entitled *Long Term Follow-Up After Administration of Human Gene Therapy Products.* |
| 4.5: Long-Term Follow-Up | Removed years 6-15 of long term follow up phase per updated FDA guidance. | Changes made per January 2020 FDA guidance entitled *Long Term Follow-Up After Administration of Human Gene Therapy Products.* |
| 8.4: Definition of a SAE | Added language clarifying that a prescheduled, elective procedure, or a routinely scheduled treatment that requires hospitalization is NOT considered to be an SAE. | Updated language to match new protocol template |
| 8.8: Reporting Timeframes | - Removed row that indicated a 5 business day requirement for a written report of other suspected adverse reactions that do not meet the definition of criteria.<br>- Updated to match language in template | Written report not required in these instances. |
| 9.4.2: Risk Assessment | Updated to include risks seen to-date in the study. | Updated to include risks seen to-date in the study. |

## LIST OF FIGURES



## LIST OF TABLES

**ABBREVIATIONS AND DEFINITIONS OF TERMS**

| | |
|---|---|
| AAV | Adeno-associated virus, a single-stranded DNA parvovirus. AAV has been engineered for use as a gene delivery vector |
| AAV2-hCHM | Adeno-associated virus vector, serotype 2, containing the normal human choroideremia gene, encoding the human REP-1 protein (Rab escort protein 1) |
| AAV2-hRPE65v2 | Adeno-associated virus vector, serotype 2, containing the human RPE65 cDNA, used in a Phase 1 and follow-on clinical trials, as well as a Phase 3 clinical trial from this Sponsor |
| ACAID | Anterior chamber-associated immune deviation |
| AE | Adverse event |
| BLA | Biologics License Application |
| C | Centigrade |
| CβA | Chicken beta actin, a promoter |
| CBC | Complete blood count |
| CCMT | Center for Cellular and Molecular Therapeutics (at CHOP) |
| cDNA | Complementary deoxyribonucleic acid |
| *CHM* | Choroideremia gene, which encodes Rab escort protein 1 (REP-1).<br>CHM in this protocol denotes choroideremia, the disease. |
| CHOP | The Children's Hospital of Philadelphia |
| CLIA | Clinical Laboratory Improvement Amendments |
| CMV | Cytomegalovirus |
| CNS | Central nervous system |
| CRF | Case Report Form |
| CTA | Clinical Trial Agreement |
| CVC | Clinical Vector Core (CCMT at CHOP) |
| db | Decibel |
| dL | Deciliter |
| DLT | Dose-limiting toxicity |
| DNA | Deoxyribonucleic acid |
| DSMB | Data Safety Monitoring Board |
| ELISpot | Enzyme-linked immunosorbent spot assay |
| ERG | Electroretinogram |
| ETDRS | Early Treatment of Diabetic Retinopathy Study (widely used visual acuity chart, first used to test visual acuity in a diabetic retinopathy study) |
| FDA | U.S. Food and Drug Administration |
| g | Gram |
| GGTase | Geranylgeranyltransferase |
| GLP | Good Laboratory Practice |
| GMP | Good Manufacturing Practice |
| Hg | Mercury |
| HIPAA | Health Insurance Portability and Accountability Act |
| HIV | Human immunodeficiency virus, causative virus of AIDS |
| hRPE | Human retinal pigment epithelium |
| IBC | Institutional Biosafety Committee |
| ICH | International Conference on Harmonisation |
| IND | Investigational New Drug |
| IOP | Intraocular pressure |

| | |
|---|---|
| IRA | Independent Review Authority |
| IRB/IEC | Institutional Review Board/Independent Ethics Committee |
| ITR | Inverted Terminal Repeat |
| IV | Intravenous |
| kDa | Kilodalton |
| L | Liter |
| LTFU | Long-Term Follow-Up |
| m | Meter |
| mg | Milligram |
| min | Minute |
| mL | Milliliter |
| mm | Millimeter |
| $mm^2$ | Square millimeter |
| mM | Millimolar |
| MOI | Multiplicity of infection |
| ms | Milliseconds |
| MTD | Maximum tolerated dose |
| ng | Nanogram |
| NHP | Non-human primate |
| NIH | U.S. National Institutes of Health |
| nm | Nanometer |
| OBA | Office of Biological Activities (of NIH) |
| OCT | Optical coherence tomography (biomicroscopy) |
| PBMC | Peripheral blood mononuclear cells |
| PCR | Polymerase chain reaction |
| QOL | Quality of life, questionnaire |
| rAAV | Recombinant adeno-associated viral vectors |
| RAC | Recombinant DNA Advisory Committee (of the NIH, OBA) |
| REP-1 | The choroideremia gene (*CHM*) encodes the Rab escort protein-1 (REP-1), deficient in choroideremia, and important in intracellular vesicular transport |
| RPE | Retinal pigment epithelium |
| RPE65 | Retinal pigment epithelium 65 kDa protein. In this protocol "RPE65" also refers to those clinical studies using the AAV2-hRPE65v2 vector for inherited retinal degeneration due to autosomal-recessive *RPE65* gene mutations |
| *RPE65* | Retinal pigment epithelium 65 kDa protein gene |
| RT-PCR | Reverse transcriptase polymerase chain reaction |
| SAE | Serious adverse event |
| SOP | Standard operating procedure |
| µL | Microliter |
| µM | Micrometer |
| vg | Vector genomes (of AAV vector) |
| WNL | Within normal limits |

## Protocol Synopsis

| | |
|---|---|
| **Study Title** | A Phase 1/2 Safety Study in Subjects with CHM (Choroideremia) Gene Mutations Using an Adeno-Associated Virus Serotype 2 Vector to Deliver the Human CHM Gene [AAV2-hCHM] to the Retina |
| **Sponsor** | Spark Therapeutics, Inc. |
| **Clinical Phase** | Phase 1/2 |
| **Study Rationale** | Gene delivery vectors based on Adeno-Associated Virus (AAV) have been in development for nearly three decades (Samulski *et al.,* 1982; Samulski *et al.,* 1987). Numerous non-clinical research studies in mice, dogs and non-human primates have demonstrated safety and efficacy of AAV vectors encoding a variety of transgenes for a number of different conditions, as well as clinical studies encompassing a wide spectrum of diseases (Mingozzi and High, 2011). Over the past several years, the first clinical trials in humans using AAV vectors for retinal disease have shown remarkable results, for both safety and clinical outcomes. Groundbreaking studies of AAV vectors by investigators at several institutions for inherited retinal degeneration due to autosomal-recessive mutations in the human retinal pigment epithelium 65 kDa protein gene (*RPE65*), have preliminarily demonstrated safety, tolerability, as well as efficacy for several endpoints initially in Phase 1 studies employing unilateral administration, and then in follow-on studies injecting the previously uninjected, contralateral eye (Maguire *et al.,* 2008; Bainbridge *et al.,* 2008; Hauswirth *et al.* 2008; Maguire *et al.,* 2009; Bennett *et al.,* 2012). The twelve subjects entered to the Phase 1 clinical trial conducted under U.S. Food and Drug Administration (FDA) Investigational New Drug (IND) #13408 generally showed improvement in light sensitivity and other visual parameters. While these early Phase 1 and follow-on studies of inherited retinal degeneration due to autosomal-recessive *RPE65* gene mutations have progressed to a Phase 3 clinical trial, leading to product approvals in the United States and Europe (LUXTURNA™), other retinal genetic diseases are being considered for gene transfer using AAV vectors encoding therapeutic proteins.<br><br>Choroideremia is a degenerative retinal disease for which gene transfer research is in progress, including a clinical trial in the United Kingdom (MacLaren *et al.,* 2014). This X-linked disease of males is characterized by deletions or mutations in the |

choroideremia gene (*CHM*) at Xq21.2, resulting in defective or absent Rab escort protein-1 (REP-1), the encoded protein of the *CHM* gene (Nussbaum *et al.,* 1985; Seabra *et al.,* 1993; Seabra *et al.,* 1995; Cremers *et al.,* 1994; Alexandrov *et al.,* 1994). Normally, Rab proteins are escorted by REP-1 to Rab geranylgeranyltransferase (Rab GGTase), which attaches geranylgeranyl groups to Rab proteins at the carboxy terminal ends (prenylation), an essential process that activates Rab proteins for their role in intracellular vesicular transport (Alexandrov *et al.,* 1994). In this way, REP-1 binds to newly synthesized Rab proteins, presents them to Rab GGTase, and delivers the geranylgeranylated Rab proteins to their target membranes. Absence or deficiency of REP-1 due to deletions or mutations in the *CHM* gene leads to cellular apoptosis and degeneration of the retinal pigment epithelium (RPE), choroid, and retinal photoreceptors. Although in normal retinas, the *CHM* gene is expressed in multiple cell types, including retinal pigment epithelium, photoreceptors and choroidal cells, there is evidence that the RPE cell is the primary disease-causing cell type. This evidence stems from observations in conditional knockout mice (Tolmachova *et al.,* 2006; Tolmachova *et al.,* 2006) and also from studies evaluating efficacy of lentivirus-mediated gene transfer (which targets RPE cells preferentially). The AAV serotype 2 vector, which targets RPE cells primarily (and other retinal cell types secondarily) is thus an ideal vector for choroideremia. Unlike lentivirus, AAV2 carries a very low risk of insertional mutagenesis.

Clinically, choroideremia is diagnosed in affected males who manifest night blindness in childhood, followed by progressive constriction of visual fields, usually symptomatic in their teenage years, and eventual total blindness. The pathological hallmarks of the disease are degeneration of the choroicapillaris, as well as retinal pigment epithelium and photoreceptors (MacDonald *et al.,* 2009). The disease course is variable, with early manifestations of decreased dark adaptation, progressing to decreased peripheral vision, followed eventually by loss of central vision, generally occurring later in life. In some patients, blindness occurs after a prolonged course. The rationale for a gene transfer approach for choroideremia is that a corrective gene delivered to the RPE early enough in the clinical course may halt degeneration and restore the RPE, retinal vasculature, and photoreceptors.

This clinical study proposes to deliver the normal human *CHM* gene (hCHM) to the subretinal space using AAV2-hCHM, a single-stranded AAV vector, based on several considerations. Previous clinical results from FDA Biologics License Application (BLA) application #125610 (IND #13408) demonstrated that following

subretinal administration of the AAV2-hRPE65v2 vector, stabilization or improvement of light sensitivity and visual endpoint measures occurred in each of the twelve participating subjects (Maguire *et al.,* 2008; Maguire *et al.,* 2009; Bennett *et al.,* 2012). When tested in cell lines *in vitro* and in mice at doses similar to those used for AAV2-hRPE65v2, the AAV2-hCHM vector showed robust levels of transgene expression as judged by the number of cells transduced and the relative level of expression of the transgene as assessed by immunofluorescence, prenylation activity and restoration of Rab27 trafficking to the cellular membranes of induced pluripotent cells from affected patients (Vasireddy *et al.,* 2013). Non-clinical toxicity studies conducted in animals (mice, dogs, non-human primates) have demonstrated an acceptable safety profile for AAV2 serotype vectors (Acland *et al*., 2001; Acland *et al*., 2005; Bennicelli *et al*., 2008; Bennett *et al*., unpublished data), and bio-distribution studies from these studies indicate limited spread of the AAV2-serotyped vector following subretinal administration. An AAV2-REP-1 vector has been administered to at least six subjects in the United Kingdom with initial results showing no significant untoward effects and some indication of efficacy (MacLaren *et al.,* 2014; Edwards *et al.,* 2016). These results comprising the use of AAV2-hRPE65v2 in non-clinical and clinical studies of inherited retinal degeneration due to autosomal-recessive *RPE65* gene mutations, along with non-clinical studies of the AAV2-hCHM vector both *in vitro* and in mice (Vasireddy *et al.,* 2013), support further clinical investigation.

In this regard, production and characterization of AAV vectors at the Center for Cellular and Molecular Therapeutics (CCMT) at The Children's Hospital of Philadelphia (CHOP) have been successfully completed for several vectors used in clinical gene transfer studies. Over the years, the CCMT Clinical Vector Core (CVC) has developed and implemented a number of processing steps to increase the uniformity and potential efficacy of AAV vectors. The manufacturing process for AAV vectors at the CCMT CVC typically results in a homogeneous product with substantial reduction of empty capsid particles. Reducing the empty capsid particles in the final vector product may have an advantage when delivering an AAV vector to the eye, by decreasing vector capsid exposure and potentially mitigating unwanted immune responses. In addition, substantial reduction of empty capsids ensures that cell surface receptors in the relatively restricted area of the injection bleb are not blocked by empty capsids, but rather that each vector particle that attaches to cell surface receptors carries a DNA payload.

| | |
|---|---|
| **Study Objective(s)** | **Primary**<br>The primary objective is to evaluate the safety and tolerability of subretinal administration of AAV2-hCHM, in an inter-subject group dose escalation in individuals with choroideremia. Toxicity related to the administration of AAV2-hCHM will be monitored in the eye and systemically, based on a comprehensive clinical monitoring plan.<br><br>**Secondary**<br>The secondary objectives are:<br>a) To define the dose of AAV2-hCHM required to achieve stable, or improved, visual function and functional vision in subjects with choroideremia; and<br>b) To characterize the immune responses to the hCHM transgene product (REP-1) and AAV2 capsid proteins following subretinal administration of AAV2-hCHM. |
| **Investigational Product** | [redacted]<br><br>AAV2-hCHM is an AAV serotype 2 vector containing single-stranded DNA encoding the human choroideremia (h*CHM*) gene under the regulatory control of the chicken β actin (CβA) promoter/CMV enhancer upstream of the CβA exon 1 and intron. |
| **Study Design** | Open label, non-randomized, inter-subject dose escalation safety study (Phase 1/2) of two vector doses (up to $5x10^{10}$ vg per eye (Dose Group 1) and up to $1x10^{11}$ vg per eye (Dose Group 2)) administered unilaterally, with at least 2 weeks between vector administrations to successive subjects for the first two subjects of a given dose group (study-wide) and at least 2 weeks between vector administrations per clinical site for the rest of the subjects in the given dose group. The eye with worse visual acuity will be injected, unless the subject prefers that eye, in which case the contralateral (non-preferred) eye will be injected. Five subjects will be entered to Dose Group 1 (subjects 1-5) and ten to Dose Group 2 (subjects 6-15). Dose escalation will be contingent upon Data Safety Monitoring Board (DSMB) approval following review of safety data of the prior cohort through at least a minimum of 30 days (that is, at least 30 days after the last subject of the previous cohort is injected). |
| **Subject Population** | **Inclusion Criteria** |

| | |
|---|---|
| **Eligibility Criteria:** | 1. Willingness to adhere to the clinical protocol and 5-year long-term follow-up as evidenced by written informed consent<br>2. Male at least 18 years of age<br>3. *CHM* gene mutation (confirmed by a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory)<br>4. Central visual field (VF) < 30° in at least 6 of the 24 meridians (using Goldmann perimetry III4e isopter) in the eye to be injected for the initial three subjects in Dose Group 1; central visual field (VF) < 30° in any of the 24 meridians (using Goldmann perimetry III4e isopter) in the eye to be injected for the remainder of subjects. Central visual field is defined as field contiguous with fixation, not including isolated islands.<br>5. Any evidence of functioning outer retinal cells within the central 10°, as defined by intact visual field, optical coherence tomography (OCT) evidence of preserved outer nuclear layer in the retina, or ophthalmologic evidence of intact retinal pigment epithelium, as detected by ophthalmoscopy and confirmed by fundus photography<br><br>**Exclusion Criteria**<br>1. Females and individuals less than 18 years of age<br>2. Unwilling to use barrier contraception methods for a period of four months following vector administration<br>3. Previous history of ocular inflammatory disease (uveitis)<br>4. Prior intraocular surgery within six months<br>5. Participation in a previous gene therapy research trial within one year of enrollment or participation in any other ocular gene therapy trial<br>6. Participation in a clinical study with an investigational drug in the past six months<br>7. Grossly asymmetrical disease, or other eye morbidity, which may render the contralateral eye ineffective as a control. Grossly asymmetric disease would include situations where one eye has significantly worse visual acuity (defined as a difference of more than 15 letters on an Early Treatment of Diabetic Retinopathy Study (ETDRS) chart) or significantly worse visual field (defined as a difference of > 10°) than the other eye.<br>[Note: Intraretinal fluid and/or cystoid macular edema are not exclusion criteria due to the prevalence of these findings in patients with choroideremia.] |

| | |
|---|---|
| | 8. Visual acuity < 20/200 on standard ETDRS testing in the eye to be injected<br>9. Presence of disease which may preclude the subject from participation in this trial, for example, cornea or lens disorders that impede retinal evaluation, systemic disease causing retinal changes such as diabetic retinopathy, neurologic disease which may confound study endpoints, malignancies which may be treated by local therapies (e.g., radiation) to the eye or central nervous system (CNS), or hematologic or other diseases which may complicate assessment of research outcomes; primary or acquired immune deficiency, because of the increased risk of opportunistic infections, such as CMV retinitis<br>10. Use of medications known to be neuroprotective and possibly beneficial for retinal disease (such as valproic acid) or retino-toxic (such as isotretinoin, thioridazine, chloroquine, and hydroxychloroquine) that could potentially interfere with the disease process and/or cause ocular adverse events; individuals who discontinue use of these compounds for 6 months may become eligible.<br>11. Individuals incapable of performing visual function testing, *e.g.* visual field and microperimetry testing, for reason other than poor vision<br>12. Any other condition that would not allow the potential subject to complete follow-up examinations during the course of the study and, in the opinion of the Investigator, makes the potential subject unsuitable for the study |
| **Number Of Subjects** | Up to a total of 15 evaluable subjects that receive AAV2-hCHM. |
| **Study Duration** | Each subject's participation will last for approximately two years in the active phase of the study and up to 5 years following vector administration for long-term follow up. The study is expected to be conducted over approximately 3.5 years for recruitment and study enrollment, including the two-year active phase follow-up. The long-term follow-up (LTFU) phase will be conducted with a duration of up to 5 years following vector administration. |
| **Study Phases**<br>**Screening/Baseline**<br>**Study Intervention**<br>**Follow-Up** | Screening/Baseline: Initial screening for eligibility is based primarily on documentation of the underlying *CHM* gene mutation by a CLIA-certified laboratory in an individual with choroideremia; this genetic diagnosis is typically obtained through standard clinical care prior to enrollment in a clinical study. If the screening indicates |

| | |
|---|---|
| **Long-Term Follow-Up** | potential eligibility, gene therapy informed consent is conducted prior to the extensive Baseline evaluation. The Baseline evaluation will be comprised of medical history and comprehensive visual and laboratory assessments (including confirmation of genetic diagnosis if adequate records are not available). If all of the criteria indicate study eligibility, the subject is offered the opportunity of vector administration and participation in the active phase of the study.<br><br>Study Intervention (Vector Administration): After confirmation of eligibility and subject agrees to study entry, the investigational product (AAV2-hCHM) is administered in the operating room on Day 0 via unilateral subretinal injection under general anesthesia to the subject's non-preferred eye (or the eye with worse visual acuity if the subject has no preference). The subject is maintained for up to 24 hours in a supine position following the procedure. The subject may be discharged during this 24-hour time period, if stable, provided the post-operative supine positioning can be maintained.<br><br>Active Phase Follow-Up: Analyses will include safety endpoints such as adverse events, comprehensive blood and urine laboratory tests, vector presence in blood and tears, pregnancy outcomes (in female partners of subjects), as well as ophthalmologic safety and efficacy endpoints including ophthalmic examination, and measures of visual function, functional vision, ophthalmologic imaging, and quality of life (QOL) questionnaire. The schedule of evaluations in the active phase will be conducted over an approximately 2-year period post vector administration to assess safety and efficacy of AAV2-hCHM (including the administration), comparing the findings obtained longitudinally from the injected eye with those of the uninjected eye serving as the control.<br><br>Long-Term Follow-Up: LTFU, up to 5 years following vector administration, will be conducted to characterize the clinical outcome and the type and seriousness of adverse events following the AAV2-hCHM gene transfer, as specified in the January 2020 FDA guidance document entitled, "Long Term Follow-Up After Administration of Human Gene Therapy Products." Assessments will include adverse events, history, physical and ophthalmic examinations, blood tests, urinalysis, pregnancy outcomes (in female partners of subjects) and retinal/visual function tests. All adverse events will be collected during the LTFU period and reporting will focus on those events related to the prior administration of AAV2-hCHM and the development or exacerbation of oncologic, hematologic, neurologic, and autoimmune events as specified in the November 2006 FDA guidance document entitled, "Gene Therapy Clinical Trials – Observing Subjects for Delayed Adverse Events." The LTFU phase |

| | |
|---|---|
| | seeks to provide longitudinal information, and should adverse events occur, more timely discovery and treatment. |
| **Efficacy Evaluations** | Efficacy will be evaluated primarily by assessing visual function, as measured by standard ophthalmological tests: Ophthalmologic examination, visual acuity (VA) by ETDRS testing, visual fields (VF) by Humphrey visual field testing, including foveal and macular thresholds, Goldmann visual field test, and Octopus kinetic visual field test (for subjects whose central visual field is >20° in at least 1 of the 24 meridians at the Screening/Baseline visit using Goldmann perimetry III4e isopter), as well as central field testing, reading speed, contrast sensitivity, and color vision testing. Additional efficacy endpoints will include: microperimetry, full-field light sensitivity threshold testing, and quality of life (QOL) questionnaire. Anatomical structures will be imaged by fundus photography (including low intensity autofluorescence) and by OCT. |
| **Pharmacokinetic Evaluations** | There will be no pharmacokinetic evaluations. However, there will be assessment of post-administration vector shedding (see Safety Evaluations, below). |
| **Safety Evaluations** | This is primarily a safety study to determine whether subretinal administration of AAV2-hCHM is safe for use in subjects with choroideremia. Adverse event collection and comprehensive laboratory safety evaluations will be conducted at regular intervals to assess safety. Viral vector administration may result in immune responses against the vector transgene product (REP-1 protein) or the AAV2 capsid; responses will be evaluated. Pregnancy outcomes (in female partners of subjects) will be assessed. If there is no limiting toxicity, up to 15 subjects will be entered at the two different dosage groups. Safety tests will include those for the presence of vector in tears and blood (vector shedding). |
| **Statistical And Analytic Plan** | Because of the small sample size of the proposed study, the statistical power of the data is limited. The statistical plan will be based primarily on safety and tolerability of the vector, and secondarily on efficacy (see comments under 'Safety Evaluations', above). Determining limiting toxicity will be the primary statistical endpoint. |
| **Data And Safety Monitoring Plan** | Monitoring gene therapy studies occurs at different levels of regulatory oversight, by several governing agencies. The day-to-day |

| | |
|---|---|
| | assessment of safety will be the responsibility primarily of the PI. An independent DSMB will oversee data and safety monitoring, with interval meetings based on DSMB guidelines. Any serious adverse event will be reported in the appropriate time-frame to the Sponsor, and as required to each of the regulatory agencies (FDA, DSMB, institutional review board (IRB) and institutional biosafety committee (IBC)). |

| **Table of Clinical Assessments** | **Day** | | | | | | | | | | **Year** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Evaluations Follow Signing of Informed Consent Form** | **Screening/ Baseline** Within 90 days of Day 0 | 0 | 1 | 3[1] | 7[1] | 14 | 30 | 90 | 180 | 365 | 1.5 | 2 | 2.5 | 3-5 |
| (Acceptable window for visit, ± Days) | | | | +1 | | ± 2 | ± 5 | ± 30 | ±30 | ±30 | ±30 | ±60 | ±60 | ±60 |
| Sequence Analysis of *CHM* gene (if not previously available), may be more than 90 days prior to Day 0 | X | | | | | | | | | | | | | |
| History and physical exam | X | | | | | | | | | X | | X | | X |
| Vital Signs | X | X | X | X | X[1] | X | | | | | | | | |
| Hematology | X | X | X | X | | X | X | X | | X | | X | | X |
| Chemistry | X | X | X | X | | X | X | X | | X | | X | | X |
| Virology | X | | | | | | | | | | | | | |
| AAV antibodies | X | | | | | | X | X | | X | | X | | |
| PBMC Collection | X | | | | | | X | X | | X | | X | | |
| Peripheral blood and tear PCR[2] | X | X | X | X | | X[2] | X[2] | X[2] | X[2] | X[2] | X[2] | X[2] | | |
| Urinalysis | X | X | X | X | | X | | | | X | | X | | X |
| Ophthalmic Examination[1] | X | | X | X[1] | X[1] | X | X | X | X | X | X | X | X | X |
| Visual Acuity | X | | X | X | X[1] | X | X | X | X | X | X | X | X | X |
| Visual Field Test, Humphrey | X | | | | | | X | X | X | X | X | X | X | X |
| Visual Field Test, Goldmann | X | | | | | | | | | | | X | | X |
| Visual Field Test, Octopus[4] | X[4] | | | | | | X[4] | X[4] | X[4] | X[4] | X[4] | X[4] | X[4] | X[4] |
| Reading Speed | X | | | | | | X | X | X | X | X | X | X | X |
| Contrast Sensitivity | X | | | | | | X | X | X | X | X | X | X | X |
| Color Vision Test | X | | | | | | X | X | X | X | X | X | X | X |
| Microperimetry | X | | | | | | X | X | X | X | X | X | X | X |
| Full-Field Light Sensitivity Threshold Testing | X | | | | | | X | X | X | X | X | X | X | X[4] |
| Fundus Photography (with low intensity autofluorescence) | X | | | | | | X | X | X | X | X | X | X | X |
| OCT | X | | | | | | X | X | X | X | X | X | X | X |
| Quality of Life Questionnaire | X | | | | | | X | X | X | X | X | X | X | X |
| **Administration of AAV2-hCHM[3]** | | X | | | | | | | | | | | | |
| AE Recording | X | X | X | X[1] | X[1] | X | X | X | X | X | X | X | X | X |
| Partner Pregnancy Outcome Recording | | | | | | | | | | X | X | X | X | X |
| Concomitant Medication Recording | X | X | X | X[1] | X[1] | X | X | X | X | X | X | X | X | X |

Notes for Table of Clinical Assessments:

[1] Day 7 evaluations, including an additional ophthalmic exam, will only be conducted if ocular inflammation is present at the Day 3 visit

[2] Blood/tear polymerase chain reaction (PCR) will be continued until two consecutive specimens test negative: Tear and blood collection will occur at each study visit until this result is obtained

[3] Day 0 = Day of vector administration: Unilateral subretinal dosing of AAV2-hCHM is conducted in the subject's non-preferred eye (or the eye with worse visual acuity if the subject does not have a preference) starting sequentially first with the Dose Group 1 (subjects #1-5, up to $5x10^{10}$ vg/eye) followed by Dose Group 2 (subjects #6-15, up to $1x10^{11}$ vg/eye). It should be noted that the degenerative component of the disease may preclude delivery of the full dose, in which case the dose given will be based on vector concentration and total volume administered (up to 300 µL).

[4] Octopus kinetic visual field test will only be done to subjects whose central visual field is >20° in at least 1 of the 24 meridians at the Screening/Baseline visit using Goldmann perimetry III4e isopter.

# 1 BACKGROUND INFORMATION AND RATIONALE

## 1.1 Introduction

### 1.1.1 Choroideremia and Gene Therapy

Choroideremia is a degenerative X-linked retinal disease characterized by deletions or mutations in the choroideremia (*CHM*) gene at Xq21.2 (OMIM gene locus #300390) resulting in defective or absent Rab escort protein-1 (REP-1), the protein encoded by the *CHM* gene (Seabra *et al.,* 1993; Seabra *et al.,* 1995; Cremers *et al.,* 1994; Alexandrov *et al.,* 1994). Normally, Rab proteins are escorted by REP-1 to Rab geranylgeranyltransferase (Rab GGTase), which attaches geranylgeranyl groups to Rab proteins at the carboxy terminal ends (prenylation), an essential process that activates Rab proteins for their role in intracellular vesicular transport (Alexandrov *et al.,* 1994). In this way, REP-1 binds to newly synthesized Rab proteins, presents them to Rab GGTase, and delivers the geranylgeranylated Rab proteins to their target membranes.

Absence or deficiency of REP-1 due to deletions or mutations in the *CHM* gene leads to cellular apoptosis and degeneration of the retinal pigment epithelium, choroid, and retinal photoreceptors. In normal retinas, the *CHM* gene is expressed in multiple cell types, including retinal pigment epithelium, photoreceptors and choroidal cells, even though there is evidence that the RPE cell is the primary disease-causing cell type. This evidence stems from observations in conditional knockout mice (Tolmachova *et al.,* 2006; Tolmachova *et al.,* 2012) and from studies evaluating efficacy of lentivirus-mediated gene transfer (which targets RPE cells preferentially) (Tolmachova *et al.,* 2012). The AAV serotype 2 vector, which targets RPE cells primarily (and other retinal cell types secondarily) is thus an ideal vector for choroideremia. Unlike lentivirus, which typically integrates into genomic DNA, AAV2 remains predominantly as a stable episome over the long-term (McCarty *et al.,* 2004; Mingozzi and High, 2011). Because it is predominantly extra-chromosomal, *in vivo* results indicate that AAV vectors carry a low risk of insertional mutagenesis (Li *et al.,* 2011; Donsante *et al.,* 2007).

Clinically, choroideremia is diagnosed in affected males who manifest night blindness in childhood, followed by progressive constriction of visual fields, usually symptomatic in the teenage to young adult years, and eventual total blindness. The pathological hallmarks of this X-linked disease, with a population prevalence of approximately 1:50,000-1:100,000 (Foundation Fighting Blindness, 2012; Genetics Home Reference, 2008; Orphanet, Choroideremia; Weckerle, Encyclopedia of Molecular Mechanisms of Disease) are degeneration of the choroicapillaris, as well as retinal pigment epithelium and photoreceptors (MacDonald *et al.,* 2009). The disease course is variable, with early clinical manifestations of decreased dark adaptation, progressing to decreased peripheral vision, followed eventually by loss of central vision, generally occurring later in life. In some patients, blindness occurs after a prolonged course.

Choroideremia is a disease candidate for gene transfer for the same reasons that other inherited retinal degenerative diseases are candidates for AAV-mediated vector delivery: a) The small and localized nature of the target area, namely the retina, with a radius of approximately 15 mm; b) the circumscribed environment of the target organ, the eye; c) the tissue boundaries

surrounding the area to be injected, which minimizes exposure of other cells to the vector and allows effective treatment with a relatively small vector dose; and, d) the favorable immune status of the eye with respect to viral vector-mediated gene transfer (Anand *et al*., 2002). Immune regulation processes in the eye can be so effective that antigens encountered in the eye potentially result in a specific tolerization known as anterior chamber-associated immune deviation (ACAID) (Streilein *et al.,* 1980).

The justification for a gene transfer approach for choroideremia is that a corrective gene delivered to the retina and RPE early enough in the clinical course may halt further degeneration of the RPE, retinal vasculature and photoreceptors. If this hypothesis is correct, visual stability or improvement may result from gene delivery (MacLaren *et al.,* 2014; Edwards *et al.,* 2016). Based on the preliminary data from gene therapy studies conducted by investigators in the U.S. and EU using an AAV2-serotype vector for inherited retinal degeneration due to autosomal-recessive *RPE65* gene mutations (LUXTURNA™, FDA BLA #125610; Bainbridge *et al.,* 2008; Hauswirth *et al.,* 2008; Maguire *et al.,* 2008; Maguire *et al.,* 2009; Simonelli *et al.,* 2010; Bennett *et al.,* 2012; Jacobson *et al.,* 2012; Cideciyan *et al.,* 2013; Bennett *et al.,* 2016), this prospect seems feasible for choroideremia.

### 1.1.2 Biology of Adeno-Associated Virus (AAV) Vectors

Adeno-associated virus is a non-enveloped, replication-defective parvovirus that has not been associated with human disease. AAV vectors are derived from the parent virus by removing all of the viral elements except for the inverted terminal repeats (ITR) and inserting the gene or genes of interest and their associated regulatory elements (Samulski *et al.,* 1982; Samulski *et al.,* 1987). The long-term safety of these vectors in humans is unknown; however, AAV vectors have been delivered to over 300 human subjects at this point, in trials for cystic fibrosis, rheumatoid arthritis, inherited retinal degeneration due to autosomal-recessive *RPE65* gene mutations, $\alpha_1$-antitrypsin deficiency, as well as hemophilia, and have been remarkably free of vector-related adverse events (Mingozzi and High, 2011). In October 2012, the European Commission granted marketing authorization for Glybera® under exceptional circumstances as a treatment for adult patients diagnosed with familial lipoprotein lipase deficiency (LPLD) confirmed by genetic testing and suffering from severe or multiple pancreatitis attacks despite dietary fat restrictions. This is the first AAV-based vector product granted marketing authorization in an ICH region, and is administered intramuscularly.

AAV vectors do not require actively dividing target cells to achieve efficient transduction, as demonstrated in post-mitotic cells of brain, muscle, liver, and retina *in vivo* (Mingozzi and High, 2011; Maguire *et al.,* 2008; Maguire *et al.,* 2009). Also, at least in animal studies, there is no immune response directed against the transduced cell, likely because all of the viral genes have been removed. In the eye, an immune-privileged site, the immune response against AAV capsid is negligible to weak (Anand *et al*., 2002), and has not precluded effective transgene expression following subsequent administration to the contralateral eye (Bennett *et al*., 2012). This absence of immune response accounts, at least in part, for prolonged (months to years) transgene expression observed in animals following a single administration of an AAV vector. Several groups have established that AAV efficiently transduces retinal pigment epithelium following a single administration into the subretinal space, resulting in long-term, (11 years in animals, > 6 years in humans), dose-dependent transgene expression (Bennett *et al.*, 2012;

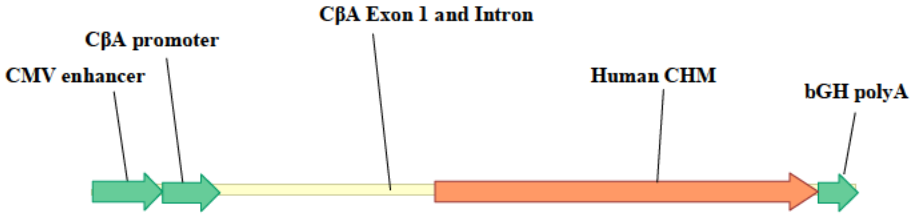
Cideciyan *et al.,* 2013). This finding supports the anecdotal results of long-term (up to 10 years) expression in human skeletal muscle, targeted in earlier clinical gene transfer studies of hemophilia (Manno *et al.,* 2003; Jiang *et al.,* 2006; Buchlis *et al.,* 2012).

### 1.1.3 Choroideremia as a Model for Gene Therapy

Retinal degenerative diseases such as choroideremia are rational targets for gene therapy using AAV. First of all, the use of an AAV2 pseudotyped vector has shown safety and utility in both animal models and human subjects with autosomal-recessive *RPE65* gene mutations (Acland *et al.,* 2001; Acland *et al.,* 2005; Maguire *et al.,* 2008; Bainbridge *et al.,* 2008; Hauswirth *et al.,* 2008; Maguire *et al.,* 2009; Amado *et al.,* 2010; Mingozzi and High, 2011; Bennett *et al.,* 2012; Jacobson *et al.,* 2012; Cideciyan *et al.,* 2013) providing impetus to develop research programs for other retinal degenerative diseases that have no currently effective treatment. Since the degenerative changes of choroideremia generally progress slowly over time, the results of unilateral vector administration can be assessed and verified, when compared to the uninjected control eye. The subretinal space has been shown to provide a favorable environment for AAV to deliver a normal gene to the RPE layer (Bennett *et al.,* 1999; Acland *et al.,* 2001; Jacobson *et al.,* 2006b; Maguire *et al.,* 2008; Bainbridge *et al.,* 2008; Hauswirth *et al.,* 2008; Maguire *et al.,* 2009; Amado *et al.,* 2010), and the limited size of the target allows for efficient transduction of retinal cells. Both *in vitro* and *in vivo* results demonstrate that the REP-1 protein is translated at levels potentially sufficient to result in a clinically relevant change within the eye (see sections 1.4.1 and 1.4.2, below). Importantly, since high levels of the transgene protein do not appear to be toxic (Tolmachova *et al.*, 2012), over-expression of REP-1 protein that may potentially occur in the context of gene therapy is not predicted to be harmful. Finally, since the eye is immune-protected, the relative lack of an immune response against the capsid protein of AAV2 and against the transgene protein may provide the ideal milieu for advantageous long-term transgene expression.

## 1.2 Name and Description of Investigational Product

The investigational product is AAV2-hCHM, an adeno-associated viral vector pseudotyped with AAV2 capsid, and the human *CHM* gene open reading frame under the regulatory control of the hybrid chicken β actin (CβA) promoter/CMV enhancer upstream of CβA exon 1 and intron (Figure 1). AAV2-hCHM is produced according to good manufacturing practice (GMP) guidelines, with substantial removal of empty capsid particles from the final product. The investigational product AAV2-hCHM will be administered by unilateral administration into the subretinal space of eligible, male subjects with choroideremia due to mutations in the *CHM* gene.




**AAV2-hCHM vector**

**Figure 1: Schematic of the AAV2-hCHM vector expression cassette**

### 1.2.1 Rationale for choice of AAV2-hCHM

AAV vectors have shown excellent efficiency of transduction of retinal pigment epithelium cells in rodents, dogs, and non-human primates following subretinal vector delivery (Acland *et al.,* 2001; Acland *et al.,* 2005; Amado *et al.,* 2010; Mingozzi and High, 2011). Additionally, recent data from clinical studies of AAV gene transfer for RPE65 deficiency from several investigational sites has shown that the AAV vector system is safe, well tolerated and efficacious (Maguire *et al.,* 2008; Bainbridge *et al.,* 2008; Hauswirth *et al.,* 2008; Maguire *et al.,* 2009; Bennett *et al.,* 2012; Jacobson *et al.,* 2012; Cideciyan *et al.,* 2013). These initial results have led to clinical research trials to test administration of vector to the previously uninjected eye, in follow-on studies. Administration to the contralateral, previously uninjected eye has been shown to be effective in a small group of subjects, (Bennett *et al.,* 2012) suggesting that AAV vectors can be administered to the contralateral eye, even with a delay of more than one year between injections, without deleterious immune responses in the human eye. These results have encouraged the development of AAV vectors for the study of other retinal diseases. A clinical trial for choroideremia was conducted in the United Kingdom at the University of Oxford, testing whether an AAV2-based vector delivering the hREP-1 encoding choroideremia gene is safe (www.clinicaltrials.gov NCT01461213). The initial results from six enrolled subjects indicate no vector-related toxicity of unilateral administration of a vector dose of $1x10^{10}$ vg in 100 µL volume, and preliminary indication of efficacy at six months (MacLaren *et al.,* 2014) and at 3.5 years following administration (Edwards *et al.,* 2016).

A number of measures have been adopted to increase the safety and efficacy of the AAV2 vector proposed for use in this clinical study. The insertion of an oversized λ stuffer sequence in the packaging plasmid helps preclude packaging of backbone sequences in the vector preparation. Substantially similar regulatory elements to those of the AAV2-hRPE65v2 vector used in the RPE65 trial are employed in this research study, since they have shown positive results in the RPE65 trial and demonstrate adequate levels of expression in the CHM non-clinical data. Additionally, the manufacturing process for AAV2-hCHM substantially removes empty capsid particles from the final product. This will decrease the total capsid antigen administered to the subjects enrolled in the study, likely decreasing the risk of triggering anti-capsid cytotoxic lymphocyte responses or other immune responses when administered to the eye. In addition, substantial removal of the empty capsid particles ensures that cell surface

receptors in the relatively restricted area of the injection bleb are not blocked by empty capsids, but rather that each vector particle that attaches to cell surface receptors carries a DNA payload.

## 1.3 Findings from Non-Clinical and Clinical Studies

The *in vitro* and *in vivo* pharmacology and toxicology described in the sections below demonstrate that AAV2 vectors can be safely delivered to the subretinal space, and that the vectors can transduce sufficient retinal pigment epithelium cells in the target area to result in therapeutic levels of transgene protein expression. The abundance of non-clinical, and clinical data in the sections below demonstrate the preliminary safety and efficacy findings of the AAV2-hRPE65v2 vector, an AAV2-pseudotyped vector used for inherited retinal degeneration due to autosomal-recessive *RPE65* gene mutations. This is a meaningful starting place, since the AAV2 capsid is the same for AAV2-hRPE65v2 and AAV2-hCHM, and the vector production and purification methods are essentially identical for these two vectors. The sponsor proposes review of the safety and biodistribution results of AAV2-hRPE65v2 to help support the use of the identically encapsidated AAV2-hCHM, for choroideremia. The RPE65 gene therapy data that supports this proposed CHM clinical study is contained in FDA BLA #125610 and will be outlined in this protocol.

For the proof-of-concept studies outlined in the sections below, we start with review of those for AAV2-hRPE65v2, followed by those of AAV2-hCHM. The non-clinical safety and efficacy results using AAV2-pseudotyped vectors encoding RPE65 in mice, dogs, and non-human primates have been described in a number of peer-reviewed publications (Acland *et al*., 2001; Dejneka *et al.,* 2004; Acland *et al*., 2005; Jacobson *et al.,* 2005; Jacobson *et al*., 2006a; Jacobson *et al*., 2006b; Jacobs *et al.*, 2006; Bennicelli *et al.*, 2008; Amado *et al.,* 2010), as briefly outlined in section 1.3.1. The safety and efficacy of clinical trials using the AAV2-hRPE65v2 vector are described below in section 1.3.2, including the Phase 1 clinical trial, as well as the follow-on study of administration to the contralateral eye. The initial results of AAV2-hCHM in cells and mice and the results of the pharmacology/toxicology study of AAV2-hCHM in non-human primates are summarized in Section 1.4.

### 1.3.1 Non-Clinical Studies of AAV2-hRPE65v2

#### *In Vitro* Studies Using AAV2-hRPE65

Numerous *in vitro* studies of several iterations of AAV2-hRPE65 vectors were conducted to develop the clinical vector AAV2-hRPE65v2, for retinal disease due to *RPE65* gene mutations. Initial studies of AAV transduction, using AAV-RPE65, of primary cell cultures of canine RPE cells, in which the vector was prepared, demonstrated expression of the RPE65 protein without toxicity (Acland *et al.,* 2001). To enhance safety, AAV2-hRPE65v1 was produced, a vector similar to that used in the initial proof-of-concept studies, except that the plasmid used to generate the vector was lengthened by insertion of a stuffer sequence. This modification prevents reverse packaging from the AAV inverted terminal repeats (ITRs), possible when the size of the vector plasmid backbone is less than the packaging limit of AAV. To enhance efficacy, introduction of a Kozak sequence (Kozak, 1997) and modification of the splice acceptor site were performed in the development of AAV2-hRPE65v2. Importantly, AAV2-hRPE65v2 transduction of cells *in vitro* (normal and mutant Briard RPE) resulted in specific

expression of both hRPE65 mRNA, as detected by reverse transcriptase polymerase chain reaction (RT-PCR), and of RPE65 protein as detected by immunohistochemistry. No increase in the basal apoptotic rate of either normal or mutant Briard RPE cells occurred after transduction with AAV2-hRPE65v2 or AAV-CMV-eGFP (used as a control) at any multiplicity of infection (MOI) tested ($10^2$ to $10^5$).

### *In Vivo* Studies Using AAV2-Pseudotyped RPE65

Many *in vivo* studies using AAV-RPE65 as well as studies using the current version, AAV2-hRPE65v2, showed favorable toxicity outcomes not only in animal models of disease but also in normal animals, as well as efficacy in animal models of disease.

### *In Vivo* Studies Using AAV-RPE65

Administration of AAV-RPE65 was safe and effective in a number of animal models of mice and dogs (Acland *et al.,* 2001; Dejneka *et al.,* 2004; Acland *et al.,* 2005; Jacobson *et al.,* 2006b). Biodistribution studies in dogs showed little vector outside the eye (vector in the heart and diaphragm in one dog, and a single submandibular lymph node in two others, as described in Jacobson *et al.,* 2006b), without germline transmission documented in any animal study. Subretinal injections of AAV-RPE65 (purified research-grade vector, generally containing a syngeneic open reading frame of RPE65), restored vision in *RPE65* "knockout" mice and in dogs which have naturally occurring mutations in *RPE65*. Importantly, transgene expression persisted after subretinal administration of AAV for the duration of the study and/or lifetime of animals that were treated in the laboratory, approximately 2.5 years in mice and greater than ten years in dogs. Age-dependent efficacy has been demonstrated in both mice and dogs, probably reflecting the time-course of retinal degeneration in these animals.

### *In Vivo* Studies Using AAV2-hRPE65v1

A non-clinical *in vivo* toxicity study in normal dogs using AAV2-hRPE65v1 (vector plasmid with stuffer sequence to allow less helper plasmid contamination of final vector product), designed in consultation with FDA, examined histopathology and biodistribution at early (3 week) and late (3 month) time-points. The dose utilized in this good lab practice (GLP) study ($1.5x10^{12}$ vg/eye) was 100-fold higher than the RPE65 Phase 1 low dose and 10-fold higher than the RPE65 Phase 1 high dose. No systemic toxicity or general ocular toxicity was observed in the normal dogs injected at this dose. All animals remained healthy and active through the study period. There was no evidence of systemic or CNS exposure to the vector after ocular administration. There was no evidence of abnormal systemic histopathology or cell-mediated immune response. However, focal retinal toxicity was apparent histologically at the 3-month time-point in regions exposed to the vector. This focal retinal toxicity in dogs at the 3-month time-point was addressed in further studies, after modifying the vector construct and excipient (see next section, below).

### *In Vivo* Studies Using AAV2-hRPE65v2

To summarize, following injection of AAV2-hRPE65v2 prepared by clinical grade methods at a dose of $8.25x10^{10}$ vg/eye (5.5-fold greater than the low dose of the RPE65 human trial and nearly two-fold greater than the proposed low dose of the CHM human trial), affected dogs showed improvement in visual parameters, and in marked contrast to dogs injected in the study

described above, showed only mild focal retinal toxicity. There was no evidence of systemic exposure to RPE65 protein at 5 weeks or 3 months after treatment. Antibodies to AAV2 capsid were detected at 5 weeks, but were not observed 3 months after treatment. Also, there was minimal evidence of RPE65 protein in ganglion cells and in optic nerves and photoreceptors (Bennicelli *et al.,* 2008). Importantly, in a follow-up study in Briard (affected) dogs, sequential administration of $1.5x10^{11}$ vg AAV2-hRPE65v2 to one eye then the contralateral eye was well tolerated and resulted in improvements in visual behavior in the animals (Amado *et al.,* 2010).

Additional non-clinical studies were conducted to address the focal toxicity observed in the canine study using AAV2-hRPE65v1 at the three-month time-point (noted above) and to examine safety issues with respect to slight modifications that were made to the vector sequence and formulation. The changes, which were made to optimize the delivery and performance of the vector, are detailed (Bennicelli *et al.,* 2008). Modifications included installation of a Kozak sequence at the translation start site of hRPE65 and modification of the splice acceptor site. Following detailed studies to assess possible loss and/or inactivation of vector using delivery devices, the concentration of Lutrol® F68 (also known as Pluronic F68 and Poloxamer 188) excipient was increased from 0.0001% to 0.001% (Bennicelli *et al.,* 2008). The following non-clinical toxicology studies were performed to evaluate safety and efficacy of the modified vector and formulation.

**AAV2-hRPE65v2 Toxicology Study in Non-Human Primates**

To evaluate the safety of AAV2-hRPE65v2, the vector construct used in the RPE65 clinical study, a non-clinical toxicology study examined late time-points in non-human primates (NHPs). The doses utilized in this study ($3x10^{11}$ vg and $7.5x10^{11}$ vg) are two-fold and five-fold higher than the high dose of the RPE65 Phase 1 human trial and 20-fold and 50-fold higher than the RPE65 human trial low dose cohort. The rationale for using NHPs centered on the fact that dog eyes show much higher amounts of inflammation after surgical procedures than those of primates and other animals (Acland et al., 2005); further, eyes of NHPs are more similar to those of humans than are dog eyes, as primates are the only animals that have a macula. Since there is only one amino acid difference between the RPE65 protein of humans and non-human primates, whereas there are eight differences between humans and dogs, it seemed less likely that the human RPE65 protein would be viewed as a foreign antigen in NHPs than in dogs. For both anatomical and immunological reasons, NHPs provide a more appropriate model.

Ten non-human primate eyes (5 animals) were injected at doses of 20-fold (4 eyes, $3x10^{11}$ vg per eye) and 50-fold (4 eyes; $7.5x10^{11}$ vg per eye) of the AAV2-hRPE65v2 human low dose of $1.5x10^{10}$ vg; two eyes were injected with vehicle alone. Final evaluations included ocular histopathology and biodistribution. Most eyes showed no inflammation. No retinal degeneration was apparent in any eye after necropsy by histopathology, as had been observed in the canine toxicology study at the same time-point. This three-month non-clinical study in Cynomolgus monkeys demonstrated no ocular toxicity resulting from a single subretinal injection of vehicle alone or AAV2-hRPE65v2 at 20- or 50-fold the human low dose and 2- or 5-fold the human high dose.

There was no mortality during the study. No test article-related clinical signs of systemic toxicity occurred. There were predicted effects of the surgery on ocular examination, including

mild and reversible inflammatory responses following surgery and alterations in the appearance of the retinal fundus due to subretinal injection. In two NHP eyes injected with AAV2-hRPE65v2, at the far periphery of the retina there was mild perivascular cuffing directed toward the vitreous and some inflammatory cells in the vitreous near the optic disc; the changes were minor in comparison to the focal toxicity observed in the normal dogs at the $1.5x10^{12}$ vg dose (100-fold higher than the RPE65 Phase 1 low dose and 10-fold higher than the RPE65 Phase 1 high dose). There were additional findings that were expected from the subretinal injection (dislodged and hypertrophic RPE cells), but no inflammation was observed in the subretinal space at either dose tested. At necropsy, there were two unexpected findings: 1) one animal had some nodules in its large intestine; and 2) another animal had a few adhesions between the diaphragm and the lung. The Sponsor believes that these findings were unrelated to the test article. The minimal toxicity observed in this study may reflect the lower dose utilized in the NHP study, or may have resulted from the greater sequence conservation between humans and NHP RPE65 as compared to canine RPE65.

There was no evidence of vector spread to pancreas, lung, bone marrow, kidney, testes/ovaries, brain, diaphragm, systemic lymph nodes, bone, thymus, heart, urinary bladder, stomach (cardiac, fundic and pyloric), colon, skeletal muscle, skin. As expected, intra-ocular fluids (anterior chamber fluid and vitreous) of all test article-injected eyes were strongly positive for presence of test article at the three-month time-point. Optic nerves (and optic chiasms) of the exposed eyes were often weakly positive for test article; this is likely a consequence of exposure to retinal ganglion cells, which line the inner surface of the retina and whose axons make up the optic nerve. These results are also in agreement with biodistribution results from non-clinical canine toxicology studies. An unexpected finding was that samples from spleen, and to a lesser extent liver, were mildly positive for the test article. The strength of the signal was related to dose, with weak or no signal identified at the lower dose. The non-clinical investigators speculate that this is due to migration of immune cells that might have engulfed vector or cells exposed to vector to these organs. The animals showed no significant humoral antibody response or cell-mediated immunological responses to the test agent.

Similarly, bilateral sequential administration of $1.5x10^{11}$ vg AAV2-hRPE65v2 was well tolerated in unaffected non-human primates (NHPs) that had been previously systemically exposed to AAV. Those animals showed increases in serum immunoreactivity to AAV2 after subretinal injection. This did not prevent safe administration to the contralateral eyes. There was also an increase in antibodies directed toward the AAV2 capsids in the anterior chamber fluid of the injected eye (only) after unilateral injection. Again, this had no effect on the ability to administer the AAV2 safely to the contralateral eye (Amado *et al.,* 2010).

In summary, simultaneous bilateral administration of AAV2-hRPE65v2 was well tolerated both in *RPE65* mutant dogs and in normal sighted monkeys, even at doses that are higher than those used to date in humans enrolled in the RPE65 Phase 1 study (Bennicelli *et al.,* 2008; Amado *et al.,* 2010). In addition, bilateral sequential subretinal administrations were well tolerated in dogs and monkeys, even if subsequent injections were carried out several months after the first one (Amado *et al.,* 2010).

### 1.3.2 Preliminary Clinical Data Using AAV2-Pseudotyped AAV Vectors

A number of clinical trials with an AAV2-based vector delivering the hREP-1 encoding choroideremia gene have been conducted or are currently open in the United Kingdom, Canada, United States, and Germany (www.clinicaltrials.gov NCT01461213, NCT02407678, NCT02077361, NCT02553135, and NCT02671539). The initial results from six enrolled subjects in the United Kingdom receiving $1x10^{10}$ vg indicated no clinical significant effects from detachment of the fovea and subretinal vector administration and no vector-related toxicity (MacLaren *et al*., 2014). Two patients who had low baseline best corrected visual acuity showed gain of letters with the treated eyes six months after vector administration and the retinal sensitivity in the treated eyes in all patients was correlated with the vector dose administered per $mm^2$ of surviving retina. This early improvement was sustained at 3.5 years after vector administration (Edwards *et al*., 2016). In 2018, a randomized, open label, and parallel controlled phase 3 trial (www.clinicaltrials.gov NCT03496012) was initiated in United States, Canada, Finland, Germany, Netherlands, and United Kingdom.

**Summary of human studies using AAV2-hRPE65v2**

While there is limited reports of clinical trial data of AAV vector mediated gene delivery for choroideremia (MacLaren *et al*., 2014; Edwards *et al*., 2016), there are considerable data for RPE65 using AAV2. Two Phase 1 studies were conducted for AAV2-hRPE65v2, a Phase 1 study (101) and a Phase 1 follow-on study (102), involving the same twelve study participants. AAV2-hRPE65v2 was initially tested in a Phase 1, first-in-human, open-label, dose escalation safety study of patients with inherited retinal dystrophy due to autosomal recessive *RPE65* gene mutations. The primary objective of the 101 study was to determine the safety and tolerability of gene transfer by subretinal administration of AAV2-hRPE65v2; a secondary objective was to assess both the objective and subjective clinical measures of efficacy in patients with *RPE65* gene mutations. Eleven of twelve subjects from the 101 study were transferred to the 102 study to receive the same vector administration to the contralateral eye in the 101 study; one additional subject who participated in the 101 study was not eligible for the 102 study [redacted]. As such, enrollment for the study was closed in 2013. The primary objective of the 102 study was to assess the safety and tolerability of non-simultaneous, bilateral subretinal administration of AAV2-hRPE65v2 (administration to the contralateral eye). The secondary objective was to evaluate the efficacy of administration of AAV2-hRPE65v2 to the contralateral eye, using pre-injection measurements of the eye injected as a control. At the time of initiation of the 102 study, all subjects had completed at least their Year One 101 study visit. A Phase 3, randomized controlled (301) study of AAV2-hRPE65v2 enrolled twenty-nine participants with all intervention subjects received non-simultaneous, bilateral administration of AAV2-hRPE65v2. The objectives of the 301 study are to assess the safety, tolerability, and efficacy of sequential, bilateral, subretinal administration of AAV2-hRPE65v2 to subjects with *RPE65* gene mutations. In addition to monitoring for safety and tolerability, efficacy is evaluated using a number of retinal and visual function tests. After at least one year from the baseline evaluations, nine control participants were able to cross over to receive administration of AAV2-hRPE65v2. There were no serious adverse events related to AAV2-hRPE65v2 and no deleterious immune responses observed in the Phase 3 trial, or in the earlier Phase 1 trials. These trials demonstrated, based on its presumed mechanism of action (recovery of biochemical activity of the RPE65 protein) and the broad range of mutations included in the clinical trials, AAV2-hRPE65v2 is indicated for the improvement of clinical symptoms of vision loss, including nyctalopia, in patients with confirmed biallelic *RPE65*

mutation-associated retinal dystrophies (Maguire *et al*., 2008; Maguire *et al*., 2009; Simonelli *et al*., 2010; Bennett *et al*., 2012; Bennett *et al*., 2016; Russell *et al*., 2017). AAV2-hRPE65v2 (voretigene neparvovec, trade name: LUXTURNA™) was approved by FDA on December 19, 2017 and European Commission on November 23, 2018 for treatment of inherited retinal dystrophy patients with confirmed biallelic *RPE65* mutations and who have sufficient viable retinal cells.

## 1.4 Non-Clinical Studies of AAV2-hCHM

### 1.4.1 In Vitro Pharmacology of AAV2-hCHM

Due to the lack of an animal model of CHM that mimics the human disease, *in vitro* models of the disease were developed for pharmacology studies (Vasireddy *et al.,* 2013). Two induced pluripotent stem cell (iPSC) lines (CPS1 and CPS2) were generated from two unrelated individuals with confirmed *CHM* gene mutations. The loss of REP-1 protein was confirmed by western blot analysis in both cell lines. A fibroblast cell line was subsequently generated from CPS1 and was named CPF1. These cell lines were used to evaluate the AAV2-hCHM vector for its ability to confer REP-1 activity.

Choroideremia patient-derived induced pluripotent stem cells were then transduced with AAV2-hCHM at MOIs of $1x10^2$, $1x10^3$, $1x10^4$, $1x10^5$, or $2x10^5$ vg/cell. Wild type induced pluripotent stem cells were also plated, but not transduced, and used as a control for normal REP-1 expression levels. Expression of REP-1 was evaluated by flow cytometry using an anti-REP-1 antibody. A qualitative comparison between untransduced wild type cells and CHM cells transduced with AAV2-hCHM at an MOI of $1x10^5$ showed similar levels of REP-1 expression. Transduction of all three cell types (CPS1, CPS2 and CPF1) with AAV2-hCHM at an MOI of $2x10^5$ vg/cell resulted in the expression of REP-1 protein (Vasireddy *et al.,* 2013), which was primarily localized to the cytoplasm. Additional studies were performed to determine if the REP-1 protein encoded by the AAV2-hCHM vector had biological activity. REP-1 plays a key role in the post-translational lipid modification of Rab small GTPases (Rabs), a process called prenylation. In addition, REP-1 is required for escort of prenylated Rab proteins to their target membranes. Thus, to assess the biological activity of REP-1 expressed from AAV2-hCHM, assays were employed to determine if Rab proteins were prenylated and subsequently escorted to the cell membrane. When CPF1, CPS1 and CPS2 cells were transduced with AAV2-hCHM at an MOI of $2x10^5$, the REP-1 protein was capable of prenylating its substrate (Rab27) and the amount of Rab27 associated with the cell membrane was similar to that seen in unaffected normal cells (Vasireddy *et al.,* 2013). To screen for short-term toxicity resulting from transduction with AAV2-hCHM, CHO cells were evaluated for apoptosis by TUNEL staining following infection by AAV2-hCHM at MOIs of $1x10^5$ or $2x10^5$. Neither dose of AAV2-hCHM resulted in cell death in the CHO cells. These studies demonstrate that AAV2-hCHM is capable of safely delivering a biologically active REP-1 protein.

### 1.4.2 In Vivo Pharmacology and Toxicology of AAV2-hCHM

One of the challenges in developing gene therapy for CHM is the lack of animal models that mimic the human disease. Unfortunately, the mouse model that exists for CHM does not reflect the human condition accurately and currently no other small or large animal models with

choroideremia-like disease have been identified. Thus, the safety of delivering AAV2-hCHM to the subretinal space was evaluated in normal wild-type mice and NHPs.

**Safety in Normal Mice**

To evaluate the safety of *in vivo* expression of AAV2-hCHM, wild type mice were injected subretinally with the recombinant adeno-associated viral vectors (rAAV) at a dose of $2.7x10^{10}$ vg/retina. Three weeks post-injection, retinal tissues were collected for protein analysis and histologic analyses. Immunoblot analysis of the retinal tissue from these animals confirmed the presence of REP-1 protein of the expected size and immunofluorescence analyses of the retinal sections confirmed the expression and localization of REP-1 protein to the inner segment and outer nuclear layers of photoreceptors and to RPE cells. The number of rows of nuclei in the outer nuclear layer was similar in treated and control mice indicating that there were no short-term degenerative changes in photoreceptors resulting from continuous expression of REP-1. In addition, in mice injected with AAV2-hCHM, retinas obtained 3 weeks after subretinal injection showed no increase in TUNEL positivity, confirming no cell death. There was also no evidence of inflammatory infiltrate in these murine tissues as judged by hematoxylin and eosin staining. Thus, our studies using AAV2-hCHM in mice have demonstrated that after subretinal injection, the *CHM* transgene is expressed in both RPE cells and in photoreceptors and no toxicity was observed (Vasireddy *et al.,* 2013).

In summary, delivery of AAV2-hCHM results in the expression of REP-1 in CHO and patient-derived iPS cells *in vitro,* and delivery to retinal cells *in vivo* results in no significant evidence of toxicity. The non-clinical data indicate that the REP-1 expressed from AAV2 in patient-derived iPS cells is able to prenylate its substrate and restore significant trafficking of Rab27 protein to the surface of the cell.

**Safety in Non-Human Primates**

A toxicology study was designed with assistance of FDA CBER representatives at a pre-IND meeting held March 11, 2013. The study was designed to include twelve (12) male non-human primates (NHP, Macaca fascicularis, Cynomolgus monkeys) for injection of either vehicle or AAV2-hCHM at two different doses and two routes of administration. GMP-process comparable AAV2-hCHM vector or vehicle was injected using the same volume (approximately 300 µL per retina), as well as the same injection device proposed for the clinical study. Each animal was injected bilaterally with either vehicle or vector. The material injected included: (a) Vehicle alone subretinally into each eye of two NHPs; (b) AAV2-hCHM at $3x10^{11}$ vg subretinally into each eye in four NHPs (a dose which is 6-fold greater than the proposed starting Dose Group 1 for the human CHM trial, and 3-fold greater than that of Dose Group 2 of the human CHM trial; (c) AAV2-hCHM at $7.5x10^{11}$ vg per retina, into each eye in four NHPs (a dose which is 15-fold greater than the proposed starting Dose Group 1 for the human CHM trial, and 7.5-fold greater than that of Dose Group 2 of the human CHM trial; and (d) AAV2-hCHM at $7.5x10^{11}$ vg into each eye injected intravitreally in two NHPs, to provide a ”worst case scenario’ for the complications of injection. Half of the animals in each group were sacrificed at 3 weeks and the other half at 3 months post-injection.

After ocular injections and peri-operative administration of sub-conjunctival triamcinolone and ophthalmic prednisone and gentamicin, animals were followed for in-life observations and

blood tests. Blood tests included complete blood counts (CBCs), serum chemistries, and serum for immunological parameters (humoral and cellular immune responses against AAV2 and antibodies against hREP-1). Tissue histopathology, including ocular histopathology, was performed upon sacrifice.

There were no clinical observations attributed to administration of the test article. There were no definitive test article-related changes in hematology parameters or in serum chemistries. Immunological testing of serum for anti-AAV humoral activity was as expected, showing elevations of anti-AAV2 antibodies in all but one animal receiving AAV2-hCHM; however, no cellular immune responses against AAV2 capsid were detected at any time points evaluated. A possible low-level antibody response directed against REP-1 was observed in one high dose animal, but the data are not conclusive at this time. No test article-related macroscopic or microscopic findings in non-ocular tissues were noted at necropsy on Day 21 or 91 and there were no test article-related changes in organ weights. Thus, no definitive test article-related systemic toxicities were noted following a single subretinal or intravitreal injection of AAV2-hCHM to Cynomolgus monkeys following either a 21-day or 91-day recovery period.

Ophthalmologic evaluations revealed that in the large majority of animals the subretinal injection caused large retinal bullae that resolved over the course of the study leaving focal pigmentary disturbances of the fundus and/or pigment dispersion onto the lens capsules in some animals.

Histopathological examination of the eyes in this study, including those that received vehicle, demonstrated changes in the RPE associated with the location of the subretinal injection site. Inflammation appeared to be a dose and time dependent phenomenon in most cases, but in all eyes that exhibited inflammation, it was graded as no worse than mild. Structural changes in the photoreceptors were only identified in two of four eyes injected with the high dose of vector and sacrificed at Day 91. These changes were mild and focal in nature, not affecting the entirety of the retina.

The investigative team is not certain if the mild focal loss of photoreceptors observed in two of four eyes injected with the high dose of AAV2-hCHM and sacrificed at day 91 was due to complications of the subretinal injection, the AAV2 capsid, or expression of human REP-1 in cynomolgus monkeys. Since similar and higher doses of AAV2-hRPE65v2 did not lead to photoreceptor loss when injected into the eyes of this same species of monkeys, this finding is not likely due to the AAV2 capsid. However, it is possible that it could be due to expression of a foreign transgene, as there are 18 amino acid differences between the human and cynomolgus REP-1 proteins, while there is only a single amino acid difference between the human and cynomolgus hRPE65 proteins. Thus, it is more likely that the hREP-1 protein will be viewed as a foreign antigen than hRPE65.

In the Phase 1/2 clinical trial, reaction to the human REP-1 transgene product is less likely. The investigative team believes the results obtained in the NHP study support the use of two dose levels in the proposed Phase 1/2 clinical trial, namely $5x10^{10}$ vg/eye and $1x10^{11}$ vg/eye.

In a separate NHP study, one cynomolgus monkey for each of the dose groups ($5x10^{11}$, $7.5x10^{11}$, and $2x10^{12}$ vg per eye) received AAV2-hCHM via bilateral subretinal injections (Couto, 2016 ARVO Meeting; Ignatova, 2016 ASGCT Meeting). Retinal tissues from the

vector exposed area (bleb), as well as from outside the bleb area, were harvested 30 days after the injection. Retina tissues from three uninjected NHPs were used as control in a species-specific quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR) assay. Human REP-1 expression was observed at robust levels within the bleb area and at very low levels outside the bleb area. Furthermore, analysis on the ratios of human REP-1 expression level to endogenous cynomolgus REP-1 expression level suggests that the two doses in this study, $5x10^{10}$ vg/eye and $1x10^{11}$ vg/eye, are likely to provide supraphysiologic levels of human REP-1 protein in the vector exposed area of retina.

### 1.5 Selection of Drugs and Dosages

The investigational product is [redacted] AAV2-hCHM is an AAV serotype 2 vector containing the normal human *CHM* gene encoding REP-1 (Rab escort protein-1) under the regulatory control of the hybrid chicken β-actin promoter coupled to the human cytomegalovirus (CMV) enhancer. Doses to be administered for the proposed inter-subject group dose escalation study design include Dose 1 of up to $5x10^{10}$ vg per eye and Dose 2 of up to $1x10^{11}$ vg per eye. Dose selection is based on previous *in vitro*, *in vivo* and clinical data from initial Phase 1, Phase 1 follow-on, and Phase 3 studies of the RPE65 gene therapy trial (FDA IND #13408) and from the *in vitro* and *in vivo* data for AAV2-hCHM (Sections 1.4.1 and 1.4.2), demonstrating that AAV2-hCHM-mediated delivery of REP-1 to patient-derived iPS cells is able to normalize prenylation and restore trafficking of Rab27 protein to the surface of the cell. The results of the NHP study also help guide the dose selection for this clinical trial (see summary in Section 1.4, above).

## 2 STUDY OBJECTIVES

### 2.1 Primary Objectives

The primary objectives are to evaluate the safety and tolerability of subretinal administration, in an inter-subject group dose escalation, of AAV2-hCHM in adults with choroideremia.

### 2.2 Secondary Objectives

The secondary objectives are:

a) To define the dose of AAV2-hCHM required to achieve stable, or improved visual function and functional vision in subjects with choroideremia; and

b) To characterize the immune responses to the *CHM* transgene protein product (REP-1) and AAV2 capsid proteins following subretinal administration of AAV2-hCHM.

## 3 INVESTIGATIONAL PLAN

### 3.1 General Schema of Study Design

This is an open label, non-randomized, inter-subject dose escalation safety and tolerability study (Phase 1/2) of unilateral subretinal administration of two vector doses (up to $5x10^{10}$ vg per eye and up to $1x10^{11}$ vg per eye) in up to 15 subjects with choroideremia, with at least 2

weeks between vector administrations to successive subjects for the first two subjects of a given dose group (study-wide) and at least 2 weeks between vector administrations per clinical site for the rest of the subjects in the given dose group. The first dose cohort will contain five subjects, barring any dose limiting toxicity, and dose escalation will be contingent upon DSMB review of the safety data through at least 30 days for all subjects in the prior cohort. The second dose cohort will contain up to ten subjects.

Potential subjects will be recruited from the clinics of investigative team members, from vision centers, and self-referrals via the web-based posting in clinicaltrials.gov. After description of the clinical trial to the subject, using the study consent and other approved recruitment materials as educational tools, if the subject remains interested in study participation, he will be invited to consent for the gene therapy study. The gene therapy Screening/Baseline evaluations are comprehensive, and will consist of the review of all relevant medical records and tests required to determine study eligibility, including obtaining CLIA-certified laboratory confirmation of CHM genotype (if not done previously). If the subject is found eligible for the study and interested in pursuing the intervention phase, the subject will be scheduled for vector administration conducted under general anesthesia. The surgical procedure for this proposed study is similar to that conducted in the Phase 1 and Phase 3 studies of AAV2-hRPE65v2 (FDA BLA #125610, LUXTURNA™) and is described in the Manual of Procedures.

As is always the case with subretinal injection, the operating surgeon has latitude in administration of the dose, based on the pathophysiology of the subject's retina. In choroideremia in particular, it is anticipated that some patients will have limited access for injection due to degenerative disease. For this reason, all doses are specified as "up to 300 μL," recognizing that some subjects may receive a smaller total subretinal volume than 300 μL, and as such, may not receive the total dose. This information will be captured should this occur.

Following unilateral vector administration under general anesthesia, the subjects will be followed up comprehensively for safety and efficacy endpoints for two years after injection, then up to an additional 3 years (total of up to 5 years) of long-term follow-up. Based on the pathophysiology of choroideremia, the investigative team does not anticipate the nearly immediate improvement of light sensitivity, to the extent that resulted for the RPE65 gene therapy studies (Maguire *et al.,* 2008; Maguire *et al.,* 2009; Bennett *et al.,* 2012). Additionally, the visual deterioration of choroideremia generally progresses more slowly than that associated with autosomal-recessive *RPE65* gene mutations (Rosenberg & Schwartz, 1994; Roberts *et al.,* 2002). The slower decline of visual function of choroideremia means that the use of the uninjected eye as a control for the injected eye becomes more statistically significant as sufficient time lapses between injections. Therefore, to minimize risk to subjects, administration of investigational product will be done in one eye for this study.

### 3.1.1 Screening/Baseline

Potential subjects will be referred from health professionals who care for patients with choroideremia, from clinics of investigative team members, from vision centers as well as potential self-referred subjects, and from the website posting on clinicaltrials.gov. For those patients who are interested, investigative team members will describe the clinical trial to the subject, using the IRB-approved study consent and other approved recruitment materials as educational tools. If the available clinical information indicates potential eligibility, and the

subject is interested in pursuing gene therapy, the subject will go through the consenting process, and sign informed consent for the gene therapy study, prior to initiation of Screening/Baseline evaluations. The gene therapy study Screening/Baseline evaluations are comprehensive, and will consist of the review of all relevant medical records and history, physical, ophthalmologic and laboratory evaluations required to determine study eligibility including obtaining CLIA-certified laboratory confirmation of CHM genotype (if not done previously). If all the inclusion criteria are met, and none of the exclusion criteria pertain (see section 3.4), the subject is eligible for the study. If the subject wishes to pursue the intervention phase, the subject will be scheduled for subretinal vector administration conducted under general anesthesia.

### 3.1.2 Study Intervention

As stated in section 3.1.1 above, if after all the applicable Baseline evaluations, the subject is eligible for the study and wishes to pursue the study intervention phase, he will be scheduled for subretinal administration of the investigational product. Preoperative tests will be obtained as well as anesthesia consultation. If the pre-operative tests and the anesthesia consultation confirm acceptability for general anesthesia, the subject will be taken to surgery, placed under general anesthesia, and receive one of two different doses of vector, prepared by the investigational pharmacy. If baseline tests, preoperative tests, or anesthesia consultation should not indicate eligibility, vector administration will be postponed until a date at which eligibility criteria are met, or the subject will be withdrawn from the study. The vector will be administered unilaterally, to the subretinal space, under general anesthesia according to the procedure described in the Manual of Procedures. The eye with worse visual acuity will be injected unless the subject prefers that eye, in which case the contralateral (non-preferred) eye will be injected.

Following vector administration, the subject will be kept in a supine position, observed and routinely monitored until stable. If no adverse events ensue, and the subject can maintain a supine position, the subject will be discharged during the 24-hour post-recovery period and scheduled for follow-up evaluation as an outpatient.

**Follow-up Phase**

After vector administration and assessment of tolerability, the two-year active phase of regular clinic visits is scheduled to comprehensively monitor safety endpoints and potential efficacy. The two-year active phase is reviewed in detail in Section 4. In addition, the subject will participate in LTFU for up to an additional 3 years, for a total of up to 5 years, following vector administration. The LTFU phase is described in detail in Section 4.5.

**Plans for Follow-On Study**

If the vector product is tolerated and effective in the two dosage groups over the follow-up period, the DSMB will discuss the possibility of a separate follow-on study for a second eye injection of the previously uninjected, contralateral eye.

## 3.2 Allocation to Study Groups

This is an open label, non-randomized, inter-subject dose escalation safety study (Phase 1/2) of the unilateral administration of two vector doses (up to $5x10^{10}$ vg per eye and up to $1x10^{11}$ vg per eye) in subjects with choroideremia, with at least 2 weeks between vector administrations to successive subjects for the first two subjects of a given dose group (study-wide) and at least 2 weeks between vector administrations per clinical site for the rest of the subjects in the given dose group. The Dose Group 1 will be administered vector first, followed by the Dose Group 2. Dose escalation will be contingent upon DSMB approval following review of safety data of the prior cohort through at least a minimum of 30 days (that is, at least a minimum of 30 days after the last subject of the prior cohort is injected). More than fifteen subjects may enroll (signing of the gene therapy study informed consent), to produce fifteen evaluable and injected subjects. Those subjects who are enrolled and fail to meet eligibility, or who are withdrawn prior to vector injection, can be replaced so that up to fifteen evaluable subjects are administered the investigational product.

## 3.3 Study Duration, Enrollment and Number of Sites

### 3.3.1 Duration of Study Participation

The total active phase study duration is approximately 27 months for each subject. There is an approximate 90-day period for verification of study eligibility, followed by 1 day of study intervention (vector administration), and 2 years for active phase follow-up. The initial approximately 27-month phase is followed by long-term follow-up for a period of up to 5 years after vector administration.

### 3.3.2 Total Number of Subjects Projected

Recruitment will stop when fifteen evaluable subjects are identified and have received subretinal injection. In the event that vector-related toxicity occurs at a dose level, discussions with FDA and the DSMB will ensue to determine whether additional subjects may be enrolled at this, or at a lower, dose (see Dosing, section 7.3, below).

## 3.4 Study Population (Eligibility Criteria)

### 3.4.1 Inclusion Criteria

1. Willingness to adhere to the clinical protocol and 5-year long-term follow-up as evidenced by written informed consent
2. Male at least 18 years of age
3. *CHM* gene mutation (confirmed by a CLIA-certified laboratory)
4. Central visual field (VF) < 30° in at least 6 of the 24 meridians (using Goldmann perimetry III4e isopter) in the eye to be injected for the initial three subjects in Dose Group 1; central visual field (VF) < 30° in any of the 24 meridians (using Goldmann perimetry III4e isopter) in the eye to be injected for the remainder of subjects. Central visual field is defined as field contiguous with fixation, not including isolated islands.
5. Any evidence of functioning outer retinal cells within the central 10º, as defined by intact visual field, optical coherence tomography (OCT) evidence of preserved outer

nuclear layer in the retina, or ophthalmologic evidence of intact retinal pigment epithelium, as detected by ophthalmoscopy and confirmed by fundus photography

### 3.4.2. Exclusion Criteria

1. Females and individuals less than 18 years of age
2. Unwilling to use barrier contraception methods for a period of four months following vector administration
3. Previous history of ocular inflammatory disease (uveitis)
4. Prior intraocular surgery within six months
5. Participation in a previous gene therapy research trial within one year of enrollment or participation in any other ocular gene therapy trial
6. Participation in a clinical study with an investigational drug in the past six months
7. Grossly asymmetrical disease, or other eye morbidity, which may render the contralateral eye ineffective as a control. Grossly asymmetric disease would include situations where one eye has significantly worse visual acuity (defined as a difference of more than 15 letters on an ETDRS chart) or significantly worse visual field (defined as a difference of > 10°) than the other eye

   [Note: Intraretinal fluid and/or cystoid macular edema are not exclusion criteria due to the prevalence of these findings in patients with choroideremia.]
8. Visual acuity < 20/200 on standard ETDRS testing in the eye to be injected
9. Presence of disease which may preclude the subject from participation in this trial, for example, cornea or lens disorders that impede retinal evaluation, systemic disease causing retinal changes such as diabetic retinopathy, neurologic disease which may confound study endpoints, malignancies which may be treated by local therapies (e.g., radiation) to the eye or CNS, or hematologic or other diseases which may complicate assessment of research outcomes; primary or acquired immune deficiency, because of the increased risk of opportunistic infections, such as CMV retinitis
10. Use of medications known to be neuroprotective and possibly beneficial for retinal disease (such as valproic acid) or retino-toxic (such as isotretinoin, thioridazine, chloroquine, and hydroxychloroquine) that could potentially interfere with the disease process and/or cause ocular adverse events; individuals who discontinue use of these compounds for 6 months may become eligible
11. Individuals incapable of performing visual function testing, *e.g.* visual field and microperimtery, for reason other than poor vision
12. Any other condition that would not allow the potential subject to complete follow-up examinations during the course of the study and, in the opinion of the Investigator, makes the potential subject unsuitable for the study

Subjects that do not meet all of the enrollment criteria may not be enrolled. Any violations of these criteria must be reported in accordance with Sponsor and IRB Policies and Procedures.

## 4 STUDY PROCEDURES

Subjects may participate in the interventional phase of the study after the nature and purpose of the protocol have been explained, written informed consent to participate has been voluntarily

granted, and the eligibility criteria listed in Section 3.4 have been met. See page xvi for the Schedule of Evaluations.

## 4.1 Screening/Baseline Visit

### 4.1.1 Gene Therapy Consent and Screening/Baseline Evaluations (Within 90 Days of Investigational Product Administration at Day 0)

The Screening/Baseline evaluations will be conducted only after the subject is consented for the gene therapy study. The Screening/Baseline evaluations are comprehensive and will consist of the review of all relevant medical records and history, physical, ophthalmologic and laboratory evaluations required to determine study eligibility, including obtaining CLIA-certified laboratory confirmation of CHM genotype (if not done previously). The Screening/Baseline evaluations will generally be done within 90 days of drug administration. The comprehensive tests are scheduled to take one day of testing (8 to 10 hours), but for some of the subjects the Screening/Baseline tests may take up to two days to complete all the required tests. If all the inclusion criteria are met, without any exclusion criteria (see section 3.4), the subject is eligible for the study, as assessed by the following:

- Gene therapy informed consent process (may be more than 90 days prior to vector administration)
- History (and medical chart review) and physical exam, including vital signs
- Prior and concomitant medications, with no excluding medications
- Confirmation of mutation affecting the *CHM* gene, based on CLIA-certified laboratory testing (may be more than 90 days prior to vector administration)
- Blood tests, including virology, hematology, chemistry, peripheral blood mononuclear cells (PBMCs) for enzyme-linked immunosorbent spot assay (ELISpot), and reactivity to AAV2 capsid
- Tear collection
- Urinalysis
- Ophthalmic examination
- Fundus photography (including low intensity autofluorescence)
- Optical coherence tomography (OCT)
- Quality of life questionnaire
- Visual/retinal function tests:
    - Goldmann visual fields (III4e isopter)
    - Humphrey visual field tests
    - Octopus kinetic visual field test (for subjects whose central visual field is >20° in at least 1 of the 24 meridians at the Screening/Baseline visit using Goldmann perimetry III4e isopter)
    - Visual acuity
    - Reading speed
    - Contrast sensitivity
    - Color vision test
    - Microperimetry
    - Full-field light sensitivity threshold testing

## 4.2 Study Intervention Phase

If results of the Screening/Baseline evaluations indicate eligibility, and the subject agrees to study entry, vector (the investigational product) administration will be scheduled. On the day of surgery, vital signs, chemistry, hematology, and urinalysis, will be performed, along with specimen collection for vector shedding (Section 4.2.1). If the anesthesia consult clears the subject for general anesthesia, the subject will proceed with vector administration under general anesthesia (Section 4.2.1). After vector administration (study intervention), there are regularly scheduled post-administration tests scheduled over a two-year interval to assess both safety and efficacy (Section 4.2.2), as well as for an additional up to 3 years, for a total of up to 5 years LTFU following vector administration (Section 4.5). Pregnancy outcomes will be collected after female partners of subjects consent and authorize collection of medical information. Subjects are encouraged to inform the Investigators as soon as a pregnancy occurs and when the outcome of the pregnancy is known.

The subject's non-preferred eye, or the eye with worse visual acuity if the subject has no preference, will be the one receiving the vector administration at the time of surgery. This would allow subjects to preserve the better eye in the event that the study product or procedures result in further visual deterioration. In patients with choroideremia, the visual function of each eye generally parallels that of the contralateral eye.

### 4.2.1 Vector Administration (Day 0)

Pre-Operative

- Vital Signs
- Chemistry, hematology, urinalysis
- Blood and tear collection for vector shedding
- Adverse event and concomitant medication recording
- Anesthesia consult prior to vector administration (may be done before Day 0)

Vector Administration

- Vector administration under general anesthesia

Eligible subjects will be admitted to the outpatient surgical clinic (or alternatively, hospital) on the day of AAV2-hCHM vector administration, and anesthesia consultation will be performed (on the day of procedure, or before admission). General anesthesia eligibility will be confirmed, prior to induction of anesthesia for vector injection. Vital signs, peripheral blood specimens, and tear specimen will be obtained before surgical vector administration. An intravenous (IV) catheter will be inserted into a suitable peripheral vein, according to the anesthesiologist's orders. Subjects will be taken to the operating room for induction of general anesthesia. After induction of anesthesia, the retinal surgeon will administer the protocol-specified dose of vector.

More specifically, the investigational product AAV2-hCHM will be thawed and diluted by the investigational pharmacy according to the Investigational Drug Data Sheet instructions, and kept at room temperature prior to injection. Up to approximately 0.30 mL (300 µL) of the

investigational product, containing the protocol-specified dose, will be administered to the subretinal space using an approved vitreoretinal cannula. The anesthesiologist will monitor vital signs during the procedure. On completion of the investigational product administration and fluid-air exchange, the vitreoretinal cannula will be withdrawn. Subjects will recover in post-anesthesia recovery, and remain until stable. If a subject is able to maintain a supine position, they may be discharged once stable, prior to 24 hours post-injection, after a minimum 4 to 6-hour monitoring period.

### 4.2.2 Post-Vector Administration Tests for Active Follow Up Phase

**Day 1**

- Vital signs
- Hematology, Chemistry, and Urinalysis
- Blood and tear collection for vector shedding
- Ophthalmic examination, including visual acuity
- AE and concomitant medications recording
- [Post-surgical discharge, if not already discharged on Day 0]

**Day 3**

- Vital signs
- Hematology, Chemistry, and Urinalysis
- Blood and tear collection for vector shedding
- Ophthalmic examination, including visual acuity: Note – If ocular inflammation is present at Day 3, an additional visit at Day 7 will be included
- AE and concomitant medications recording

**Day 7**

Only if ocular inflammation is present at Day 3, a Day 7 visit is added

- Vital signs
- Ophthalmic examination, including visual acuity
- AE and concomitant medications recording

**Day 14**

- Vital signs
- Hematology, Chemistry, and Urinalysis
- Blood and tear collection for vector shedding, if indicated
- Ophthalmic examination, including visual acuity
- AE and concomitant medications recording

**Day 30**

- Blood tests, including hematology, chemistry, PBMCs for ELISpot, and reactivity to AAV2 capsid

- Blood and tear collection for vector shedding, if indicated
- Ophthalmic examination
- Fundus photography (including low intensity autofluorescence)
- OCT
- QOL questionnaire
- AE and concomitant medications recording
- Visual/retinal function tests:
    - Visual acuity
    - Humphrey visual field tests
    - Octopus kinetic visual field test (for subjects whose central visual field is >20° in at least 1 of the 24 meridians at the Screening/Baseline visit using Goldmann perimetry III4e isopter)
    - Reading speed
    - Contrast sensitivity
    - Color vision test
    - Microperimetry
    - Full-field light sensitivity threshold testing

**Day 90**

- Blood tests, including hematology, chemistry, PBMCs for ELISpot, and reactivity to AAV2 capsid
- Blood and tear collection for vector shedding, if indicated
- Ophthalmic examination
- Fundus photography (including low intensity autofluorescence)
- OCT
- QOL questionnaire
- AE and concomitant medications recording
- Visual/retinal function tests:
    - Visual acuity
    - Humphrey visual field tests
    - Octopus kinetic visual field test (for subjects whose central visual field is >20° in at least 1 of the 24 meridians at the Screening/Baseline visit using Goldmann perimetry III4e isopter)
    - Reading speed
    - Contrast sensitivity
    - Color vision test
    - Microperimetry
    - Full-field light sensitivity threshold testing

**Day 180**

- Blood and tear collection for vector shedding, if indicated
- Ophthalmic examination
- Fundus photography (including low intensity autofluorescence)
- OCT

- QOL questionnaire
- AE and concomitant medications recording
- Visual/retinal function tests:
    - Visual acuity
    - Humphrey visual field tests
    - Octopus kinetic visual field test (for subjects whose central visual field is >20° in at least 1 of the 24 meridians at the Screening/Baseline visit using Goldmann perimetry III4e isopter)
    - Reading speed
    - Contrast sensitivity
    - Color vision test
    - Microperimetry
    - Full-field light sensitivity threshold testing

**Day 365**

- History and physical exam
- Blood tests, including hematology, chemistry, PBMCs for ELISpot, and reactivity to AAV2 capsid
- Blood and tear collection for vector shedding, if indicated
- Urinalysis
- Ophthalmic examination
- Fundus photography (including low intensity autofluorescence)
- OCT
- QOL questionnaire
- AE and concomitant medications recording
- Partner pregnancy outcomes recording
- Retinal/visual function tests:
    - Visual acuity
    - Humphrey visual field tests
    - Octopus kinetic visual field test (for subjects whose central visual field is >20° in at least 1 of the 24 meridians at the Screening/Baseline visit using Goldmann perimetry III4e isopter)
    - Reading speed
    - Contrast sensitivity
    - Color vision test
    - Microperimetry
    - Full-field light sensitivity threshold testing

**Year 1.5**

- Blood and tear collection for vector shedding, if indicated
- Ophthalmic examination
- Fundus photography (including low intensity autofluorescence)
- OCT

- QOL questionnaire
- AE and concomitant medications recording
- Partner pregnancy outcomes recording
- Retinal/visual function tests:
    - Visual acuity
    - Humphrey visual field tests
    - Octopus kinetic visual field test (for subjects whose central visual field is >20° in at least 1 of the 24 meridians at the Screening/Baseline visit using Goldmann perimetry III4e isopter)
    - Reading speed
    - Contrast sensitivity
    - Color vision test
    - Microperimetry
    - Full-field light sensitivity threshold testing

**Year 2**

- History and physical exam
- Urinalysis
- Blood tests, including hematology, chemistry, PBMCs for ELISpot, and reactivity to AAV2 capsid
- Blood and tear collection for vector shedding, if indicated
- Ophthalmic examination
- Fundus photography (including low intensity autofluorescence)
- OCT
- QOL questionnaire
- AE and concomitant medications recording
- Partner pregnancy outcomes recording
- Visual/retinal function tests:
    - Visual acuity
    - Humphrey visual field tests
    - Goldmann visual fields (III4e isopter)
    - Octopus kinetic visual field test (for subjects whose central visual field is >20° in at least 1 of the 24 meridians at the Screening/Baseline visit using Goldmann perimetry III4e isopter)
    - Reading speed
    - Contrast sensitivity
    - Color vision test
    - Microperimetry
    - Full-field light sensitivity threshold testing

For evaluations after Year 2, the subject will go into the long-term follow-up phase (see section 4.5). The subject returns to the clinic for follow-up at Year 2.5 and then annual follow-up, done in the clinic, for Years 3-5 for safety evaluations, standard ophthalmologic examinations, and retinal/visual function tests. The subject is questioned about general and ophthalmic health, as

well as all adverse events including the development or exacerbation of oncologic, hematologic, neurologic, or autoimmune diseases and any female partner pregnancy outcomes.

### 4.3 Prior and Concomitant Medication

All subjects will remain on their usual medications throughout the study interval. Prior medications will be recorded, through Day -30 of vector injection; concomitant medications will be recorded throughout study participation. All medications that the subject is taking will be documented, including dosage, with special note of those that may affect vision (*e.g.*, vitamin A preparations).

Prior and concomitant medications that will be recorded include prescription medications, blood products, dietary supplements/vitamins, electrolyte supplementation, and over-the-counter medications (OTC). Administration of the following will not be recorded, unless the Investigator considers this important to the assessment of an adverse event.

- Intravenous fluids
- Enteral or parenteral nutrition
- Electrolyte supplementation
- Albumin
- Oxygen
- Intra-operative medications

#### Prior Medications

A medication history will be taken during the Screening/Baseline period and updated at the time of administration of the investigational product (Day 0). Medications used within 30 days prior to vector administration, including dosage, will be entered in the case report form (CRF).

#### Concomitant Medications

Concomitant medications taken during the study period, including dosage, will be entered in the CRF.

#### Prohibited Concomitant Medications

Use of any of the following medications is prohibited from Day 0 to Year 5:

- Valproic acid
- Isotretinoin, hydroxychloroquine, chloroquine and thioridazine

### 4.4 Subject Completion/Withdrawal

Subjects may withdraw from the study at any time without prejudice to their care. As much as is feasible, the study team will follow subjects after investigational product administration since this gene transfer study offers the potential of single-use, long-term treatment that cannot be practically withdrawn. It will be documented whether or not each subject completes the clinical study, and the reason for study discontinuation including: consent withdrawn; death; lost to follow-up; protocol violation; and Investigator or Sponsor discretion (prior to investigational product administration). If the Investigator becomes aware of any serious,

related adverse events after the subject completes or withdraws from the study, the SAE will be recorded in the source documents and entered in the study CRF.

### 4.4.1 Early Termination Study Visit

If the subject withdraws after receiving the investigational product and before the Year 5 visit, every effort should be made to arrange an Early Termination Study Visit. The study procedures to be done should be identical to those of the next scheduled visit. The visit endpoints may consist of the following evaluations:

- History and physical exam
- Blood tests, including hematology, chemistry, PBMCs for ELISpot, and reactivity to AAV2 capsid
- Blood and tear collection for vector shedding, if indicated
- Urinalysis
- Ophthalmic examination
- Fundus photography (including low intensity autofluorescence)
- OCT
- QOL questionnaire
- AE and concomitant medications recording
- Partner pregnancy outcomes recording
- Visual/retinal function tests:
    - Visual acuity
    - Humphrey visual field tests
    - Goldmann visual fields (III4e isopter)
    - Octopus kinetic visual field test (for subjects whose central visual field is >20° in at least 1 of the 24 meridians at the Screening/Baseline visit using Goldmann perimetry III4e isopter)
    - Reading speed
    - Contrast sensitivity
    - Color vision test
    - Microperimetry
    - Full-field light sensitivity threshold testing

If the subject withdraws before receiving the investigational product, early termination study procedures are not necessary.

## 4.5 Long-Term Follow-Up

The LTFU phase is up to 5 years following vector administration, subjects are questioned about general and ophthalmic health, all adverse events including the development or exacerbation of oncologic, hematologic, neurologic, or autoimmune diseases, as well as any female partner pregnancy outcomes. The LTFU phase is drafted based on the January 2020 FDA guidance entitled *Long Term Follow-Up After Administration of Human Gene Therapy Products.* For Years 2.5, 3, 4, and 5 visits, subjects return to the clinic for safety evaluations, standard ophthalmologic examinations, and retinal/visual function testing.

**LTFU Visits (Years 2.5, 3 to 5)**:

- An annual (Years 3 to 5) physical exam with complete history; laboratory analysis will include:
  - Hematology (CBC with differential)
  - Chemistry panel (including liver and renal function tests)
  - Urinalysis
- Ophthalmic evaluations and retinal/visual function testing:
  - Ophthalmic examination
  - OCT
  - Fundus photography (including low intensity autofluorescence)
  - Visual acuity
  - Humphrey visual field test
  - Goldmann visual fields (III4e isopter; only annually)
  - Octopus kinetic visual field test (for subjects whose central visual field is >20° in at least 1 of the 24 meridians at the Screening/Baseline visit using Goldmann perimetry III4e isopter)
  - Reading speed
  - Contrast sensitivity
  - Color vision test
  - Microperimetry
  - Full-field light sensitivity threshold testing
  - QOL questionnaire
- Adverse event reporting, including any unexpected illness and / or hospitalization. Subjects will be encouraged to monitor themselves and to assist in reporting adverse events. All adverse events should be recorded. Documentation of adverse events should include causality (unlikely, possibly, or probably related) to the vector administration procedure, investigational product, study measures and any newly arising or exacerbated clinical events in four general disease categories; oncologic, hematologic, neurologic, and autoimmune disorders.
- Investigators will maintain in the case history records of exposures to mutagenic agents and other medicinal products along with subjects' adverse event profiles. If, according to the investigator, exposure to a mutagenic agent occurred, or a medicinal product was given, prior to and thought to contribute to an adverse event, or given as a response to an adverse event, then the mutagenic agent or medicinal product will be reported to the Sponsor via case report forms.
- Concomitant medications recording
- Partner pregnancy outcomes recording

# 5 STUDY EVALUATIONS AND MEASUREMENTS

## 5.1 Screening/Baseline and Monitoring Evaluations and Measurements

### 5.1.1 History and Medical Record Review

Review of medical records will be conducted, including review of paper and electronic records of subjects, to confirm ophthalmologic history, inclusion/exclusion criteria, and pertinent past medical history. Those items that will be abstracted from the medical chart (paper and electronic) will include age, gender, race (demographic information), relevant past ophthalmologic and medical history including visual acuity, visual fields, genotype, concomitant medications, laboratory reports, electroretinograms (ERG), fundus photographs, optical coherence tomograms, radiological and scanning results, ultrasound results, pathology reports, and other relevant clinical information. If the subject is entered on study, the medical history will be updated in a longitudinal manner, to determine if any significant events have occurred during the course of follow-up, and to document concomitant medication changes.

### 5.1.2 Physical Examination

Screening/Baseline and interim physical examinations will include measurements of vital signs as well as general physical examination. The Investigators, or qualified designee, will perform physical examinations and measure body weight and height at the time points specified in the schedule of assessments. In addition, symptom-oriented physical examinations will be performed when clinically indicated. Clinically relevant abnormalities prior to the first dose of investigational product will be recorded as medical history; clinically relevant abnormalities after the first dose of investigational product will be recorded as AEs.

### 5.1.3 Laboratory Evaluations

Blood sampling will be performed for the following standard clinical laboratory evaluations (see Table 5.1.3.1); repeat analysis and/or additional sampling and testing will be performed when clinically indicated:

- Sequence analysis of the *CHM* gene by a CLIA-certified laboratory
- Hematology (CBC and differential)
- Chemistry panel to include liver and renal function tests
- Virology testing to include HIV screen

Urine testing will be performed by standard clinical laboratory analyses (see Table 5.1.3.1); repeat analysis and/or additional sampling and testing will be performed when clinically indicated:

- Urinalysis (UA) including pH, color, and microscopic exam

Analysis will be performed for the following research laboratory evaluations (see Table 5.1.3.2):

- AAV Viral Capsid Antibody
- PBMC for ELISpot
- Whole blood and serum for PCR analysis (vector shedding)

- Tears collected for vector shedding by PCR analysis

***5.1.3.1 Table: Summary of Clinical Laboratory Tests***

| Category | Tests |
|---|---|
| *CHM* gene analysis | Sequence/mutation analysis by CLIA-certified laboratory |
| Hematology | CBC and differential |
| Chemistry panel | Standard panel per clinical laboratory (including liver and renal function tests) |
| Virology tests | Screening tests for HIV |
| Urinalysis | Standard urinalysis per clinical laboratory |

***5.1.3.2 Table: Summary of Research Laboratory Tests[1]***

| Category | Tests |
|---|---|
| AAV Viral Capsid Antibody | Measurement of antibody against AAV viral capsid, serotype 2 |
| PBMC for ELISpot | PBMC evaluation for IFN γ to evaluate activation of immune response |
| Whole blood and serum for vector sequences | Blood specimens are collected, DNA is extracted, and PCR analysis conducted |
| Tears for vector sequences | Tear specimens are collected, DNA is extracted, and PCR analysis conducted |

[1]Each test is conducted according to Sponsor or designee standard operating procedure (SOP).

Ophthalmic tests will be performed for the following standard and research evaluations (see Table 5.1.3.3):

- Ophthalmic examination
- Visual acuity
- Humphrey visual field tests
- Octopus kinetic visual field test (for subjects whose central visual field is >20° in at least 1 of the 24 meridians at the Screening/Baseline visit using Goldmann perimetry III4e isopter)
- Reading speed
- Contrast sensitivity
- Color vision test
- Microperimetry
- Full-field light sensitivity threshold testing
- Fundus photography (including low intensity autofluorescence)
- OCT
- QOL questionnaire

### *5.1.3.3 Table: Summary of Ophthalmic Tests & Evaluations*[1]

| Category | Tests |
|---|---|
| Visual Function | Ophthalmic examinations, using standard instruments and methods |
| | Visual Acuity (ETDRS) |
| | Goldmann Visual Field Test (III4e isopter) |
| | Humphrey Visual Field Tests |
| | Octopus Kinetic Visual Field Test[2] |
| | Contrast Sensitivity |
| | Color vision test |
| | Microperimetry |
| | Full-field Light Sensitivity Threshold Testing |
| Functional Vision | Reading Speed |
| Imaging | Fundus Photography |
| | Optical Coherence Tomography (OCT), to measure retinal thickness |
| Evaluation of activities of daily life | QOL Questionnaire |

[1]Each test is conducted according to standard ophthalmology office practice or Sponsor or designee SOP.
[2]Octopus kinetic visual field test will only be done to subjects whose central visual field is >20° in at least 1 of the 24 meridians at the Screening/Baseline visit using Goldmann perimetry III4e isopter.

## 5.2 Efficacy Evaluations

Evaluations will be made for visual acuity, Goldmann and Humphrey visual field tests, Octopus kinetic visual field test, reading speed, contrast sensitivity, color vision test, microperimetry, full-field light sensitivity threshold testing, fundus photography (including low intensity autofluorescence), optical coherence tomography (OCT), and QOL questionnaire. Each of these tests is either a standard clinical test done according to standard clinical practice or performed by the investigative team according to Sponsor SOP.

Visual Acuity: Visual acuity measures will document any change in central vision, the ability to resolve standard optotype images presented as optotypes/letters corresponding to different visual angles, *i.e.,* image size. This testing will use ETDRS charts. The level of central visual resolution is converted to a visual minimal angle of resolution score (LogMAR) for comparison purposes.

Visual Field Tests: Visual field parameters will evaluate alterations in function of different regions of the retina; kinetic fields will be measured with Goldmann (III4e isopter), and Humphrey static fields with computerized testing, including foveal and macular thresholds, and a central field. In addition, Octopus kinetic perimetry will be done to subjects whose central

visual field is >20° in at least 1 of the 24 meridians at Screening/Baseline visit using Goldmann perimetry III4e isopter.

Reading speed: The reading speed test will require subjects to read designated word charts with black letters on a white background aloud and subsequently, the critical print size and optimal reading speed will be calculated.

Contrast Sensitivity: Contrast sensitivity will measure the subject's ability to discern targets presented at varying levels of contrast.

Color Vision Test: Color vision testing will be conducted if the subjects are able to perceive the test objects.

Microperimetry: Microperimetry will be conducted in the dark-adapted state to evaluate maximal retinal sensitivity. Microperimetry measurements of the injected eyes will also be compared to the control (uninjected) eyes. Microperimetry will measure minimal detectable stimulus, mean sensitivity, and visual field size.

Full-field Light Sensitivity Threshold Testing: Full-field light sensitivity threshold (FST) testing measures the light sensitivity of the entire visual field by recording the luminance at which a subject reports seeing the dimmest flash. The test is carried out on subjects with dilated eyes in a dark-adapted state; subjects are seated in front of a Ganzfeld dome in which the light flashes are generated. The light sensitivity of each eye is measured separately by removing patches from one eye (and then the other). A sound is generated at the time of the light flash and the subject presses one button when they see a flash or a second button if they do not see a flash. Flashes of varying luminance (in a range spanning ~80 dB) are presented in a randomized order, except that the series starts with dim flashes. From this data, an algorithm calculates the minimum luminance (for each eye) at which the subject perceives light.

Fundus Photography: Fundoscopy will be performed with indirect ophthalmoscopic exam and fundus biomicroscopy. Photographs will be taken with a fundus camera following standard clinical methods.

Optical coherence tomography (OCT): OCT captures micrometer-resolution, three-dimensional images from within optical scattering media (*e.g.,* retinal tissue). The Heidelberg Spectralis OCT machine will be used. Optical coherence tomography is an interferometric technique, typically employing near-infrared light. The use of relatively long wavelength light allows it to penetrate into the scattering medium, providing important images, including the thickness of the retina.

Quality of Life questionnaire: The Investigators will use a QOL questionnaire relevant to the subject population.

### 5.3 Safety Evaluations

Safety will be monitored and data captured by description of adverse events and pregnancy outcomes in female partners of subjects post vector administration. Adverse events will be reported to the regulatory agencies that oversee gene therapy trials, including the Sponsor, local

IRB and IBC, FDA, NIH OBA, and the independent DSMB. The reporting details are more thoroughly described in Section 8.

# 6 STATISTICAL CONSIDERATIONS

Safety analyses will be performed for all participants that receive AAV2-hCHM. Summary statistics will be provided to evaluate efficacy endpoints by dose group for all evaluable subjects. Each subject's *CHM* gene sequence information will be obtained for information only. Because of the small sample size, it is not anticipated that any statistically significant correlation will be possible between a particular mutation in the *CHM* gene and safety and efficacy outcomes.

Efficacy data will be collected; however, because of the number of subjects and the size of the dosage groups it is likely that the study lacks sufficient power to reach statistical significance. If the study results in favorable safety and tolerability profile of AAV2-hCHM, as well as an indication of efficacy, follow-on studies will be proposed to more completely evaluate statistical significance and clinical applicability of this gene therapy approach.

# 7 INVESTIGATIONAL PRODUCT (INTERVENTION)

The investigational product is [redacted] AAV2-hCHM is an AAV serotype 2 vector containing single-stranded DNA encoding the human *CHM* gene driven by the chicken β actin (CβA) promoter/CMV enhancer upstream of CβA exon 1 and intron.

The vector production facility and methods have been characterized by the Manufacturer, CCMT CVC (Wright, 2008; Wright *et al.,* 2010; Wright & Zelenaia, 2011).

The gene therapy material to be used in this clinical study has been manufactured under current Good Manufacturing Practices (cGMP). [redacted] The final formulation of the [redacted]

## 7.1 Packaging

AAV2-hCHM is derived from a virus and should be considered and handled as an infectious agent. AAV2-hCHM is supplied as a frozen liquid at a volume of 1 mL in a 1.5 mL polypropylene sterile cryogenic screw cap vial. AAV2-hCHM is to be stored frozen at less than -60°C. Just prior to use, the frozen product will be thawed at room temperature. The syringe for AAV2-hCHM transport and administration will be prepared sterilely and filled in a bio-safety cabinet. After filling, the syringe is stored at room temperature. Within the operating room, the syringe is coupled to the surgical device. The product is to be administered within six hours of thawing to assure maximum potency. Preparation materials will be maintained by the investigational pharmacy for a period of two months; at this time, they will be destroyed or shipped back to the Sponsor, per instructions.

A qualified pharmacist with specific training on this protocol will be responsible for vector receipt from the Sponsor, storage, documentation of traceability of investigational product at the investigational site, and preparation (dilution) on the day of surgery. Prior to dispensing of the investigational product from the investigational pharmacy for administration to a study subject, a Pharmacy Order Form shall be completed and signed by a Principal Investigator or qualified Sub-Investigator. This form is to be completed on a subject-by-subject basis; the Investigators should complete and initial the date and time section. Upon receiving the order form, the investigational pharmacy will prepare the investigational product as follows:

Universal precautions and appropriate personal protective equipment will be used by investigational pharmacy personnel performing the dilution. The frozen AAV2-hCHM and diluent vials shall be thawed at room temperature and gently mixed. Appropriate volumes of the AAV2-hCHM and diluent products, both provided by the Sponsor, will then be combined using aseptic techniques in a biosafety cabinet. The Sponsor's instructions for this procedure, including the specific volumes of AAV2-hCHM and diluent to combine, will accompany each individual lot of material delivered to the investigational pharmacy.

### 7.2 Labeling

The product label indicates: Investigational product name (AAV2-hCHM); manufacturer (CCMT CVC, Children's Hospital of Philadelphia PA, USA); specific lot number; date of manufacture; vial number; storage instructions (< 60°C); and investigational product warning (Caution: New Drug – Limited by Federal Law to Investigational Use).

### 7.3 Dosing

The two doses of AAV2-hCHM proposed for this study are: Up to $5x10^{10}$ vg per eye (Dose Group 1) and up to $1x10^{11}$ vg per eye (Dose Group 2). Dosing was based on several considerations. *In vitro* non-clinical data demonstrated robust REP-1 protein levels and normalization of prenylation activity as well as restoration of Rab27 intravesicular transport to the cell membrane (see section 1.4.1) at doses comparable to those used in the RPE65 non-clinical studies, without toxicity in mice (Vasireddy *et al.,* 2013). Additionally, the data from the prior Phase 1, the Phase 1 follow-on, and the Phase 3 studies for *RPE65* mutations support safety in the dose range of $1.5x10^{10}$ to $1.5x10^{11}$ vg/eye. Because choroideremia results in progressive death of peripheral retina, some subjects enrolled in this study may not have enough viable retina to tolerate an injection volume of 300 µL. In such instances, the maximum volume of the given vector concentration will be injected and the volume will be recorded, to calculate the exact dose administered. The study in the UK of an AAV2 vector delivering REP-1 via an AAV2 vector to patients with choroideremia also supports the proposed doses. The UK starting dose was $1x10^{10}$ vg/eye in 100 µL, tailored to the area of intact retinal cells (MacLaren *et al.,* 2014); this study has progressed to a dose of $1x10^{11}$ vg/eye, with no reports of vector-related toxicity (MacLaren, Choroideremia Research Foundation Meeting, June 15-17, 2014, Denver).

Subjects who enroll and are eligible but not injected may be replaced with another eligible subject to achieve up to 15 injected, evaluable subjects. There will be a minimum two-week interval between subject injections. This interval is based on the accumulated safety data of the RPE65 clinical trials. There may be adverse events beyond the first two weeks, but these generally are complications of those adverse events arising in the first two weeks post-

administration. Barring possible or probable vector-related dose limiting toxicity in one of the dosage groups, it is anticipated that a total of 15 evaluable, injected subjects will be entered on study.

### 7.4 Stopping Rules

The toxicity criteria are based on the WHO toxicity scale, with added assessments pertaining to ocular AEs (located in the Manual of Procedures). Dose-limiting toxicity (DLT) will be defined as any irreversible Grade 3 or higher toxicity, or any reversible Grade 4 toxicity, that is possibly or probably related to AAV2-hCHM. Irreversible toxicities are defined as those that persist at the same (or higher) grade for more than seven days; reversible toxicities are those resolving in seven or fewer days.

If DLT is experienced, further subject enrollment will be delayed pending consultation with the DSMB. If a subject experiences any DLT, the DSMB will assist in determining whether additional subjects should be enrolled at the same dose level, or at a lower dose level. If two subjects of a dose group develop limiting toxicity, possibly or probably attributable to the vector, the maximum tolerated dose (MTD) will have been reached. In the event this occurs in Dose Group 2, additional subjects may be enrolled to the group just below the MTD, *i.e.* Dose Group 1.

If a single occurrence of Grade 4 toxicity, excluding toxicities covered under the category "General", or death of a subject occurs and is deemed possibly or probably related to the AAV2-hCHM, the Sponsor will halt enrollment and notify the DSMB and FDA. Further enrollment and/or vector administrations will be conducted only after FDA and the Sponsor confer to determine that the IND is not on clinical hold, and the DSMB agrees to continue enrollment. Study visits for subjects that have already received AAV2-hCHM will continue per protocol.

The Sponsor or designee will report all SAEs and AEs to the DSMB, FDA, NIH OBA, and to Principal Investigators (who will in turn notify the IRBs/Institutional Ethics Committees (IECs) of record) within the required reporting timeframes. In addition to the stopping rules outlined above and based on the toxicity criteria table (located in the Manual of Procedures), the Sponsor will respond to recommendations of the DSMB.

### 7.5 Compliance and Adherence

Following the subretinal administration of AAV2-hCHM, subjects will be followed according to the protocol schedule of evaluations. Subjects will be encouraged to follow-up completely and according to the study endpoints. Non-adherence to the protocol will be reported to the relevant regulatory groups overseeing the study.

### 7.6 Accountability

Adequate records of investigational product receipt and disposition will be maintained by the investigational Pharmacy, and records of receipts, investigational drug orders, dispensing records, and disposition forms will be examined during the course of the study. The purpose of these records is to ensure the regulatory authorities and the Sponsor that the investigational new drug will not be distributed to any person who is not a study subject under the terms and conditions set forth in this protocol. The investigational product is to be prescribed by the

Investigators or qualified designee and may not be used for any purpose other than that described in the protocol. At study completion, all investigational product supplies including partially used and empty containers must be destroyed or returned to the Sponsor, or designee, per their instructions.

# 8 SAFETY MANAGEMENT

## 8.1 Clinical Adverse Events

All adverse events will be monitored throughout the active and long-term follow-up phase following vector administration. Adverse event inquiry and assessment will be conducted on an ongoing basis during study participation through the following means:

- Subject inquiry or report
- Physical observation and/or physical examination
- Laboratory and any other diagnostic test results review
- Telephone call
- E-mail
- Postal query

The toxicity reporting scale (located in the Manual of Procedures) should be used, when possible, to assess adverse events.

## 8.2 Adverse Event Reporting

All events described by the subject will be reported as AEs, regardless of toxicity grade in.

Protocol-defined safety laboratory test results will be collected prospectively and analyzed separately as part of the study. Additional laboratory test results at other time points may be available to the Investigators as part of standard clinical practice. Throughout the active phase of the study, laboratory-related abnormalities should be recorded as AEs only if considered clinically significant, outside the range of expected values given the subject's baseline assessments and clinical course, and not known to be part of another AE diagnosis. As per regulatory guidelines, pre-existing conditions, including abnormal lab values manifest at baseline testing, will only be reported as adverse events if the condition worsens in severity and/or frequency.

The Investigators will follow an AE until the event is either resolved or assessed as stable.

The Investigators are responsible for notifying the Sponsor representative or designee and the IRB of all AEs and other unanticipated problems related to research. Unanticipated problems are other types of incidents, experiences, and outcomes that occur during the conduct of human subject research that are not considered adverse events. For example, some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased *risk* of harm, but no harm occurs.

The Sponsor or designee will notify the appropriate federal regulatory authorities (FDA, NIH OBA, DSMB). Events that do not require expedited reporting will be summarized at the time of continuing review / annual report.

### 8.3 Definition of an Adverse Event

An *adverse event* (AE) is any untoward, undesired, or unplanned clinical event in the form of signs, symptoms, disease, or laboratory or physiological observations occurring in a subject participating in a clinical study with the Sponsor's investigational product, regardless of causal relationship. Adverse events will include any conditions that: 1) were not present prior to investigational product administration, but appeared following investigational product administration; or 2) were present prior to investigational product administration, but worsened in severity and/or frequency following investigational product administration.

Adverse events will be noted in the study records and on the case report form with a full description including the nature, date of onset (as well as time, if feasible), determination of non-serious versus serious, intensity (mild, moderate, severe, life-threatening, death), duration (*i.e.* end date and time, if feasible), causality, and outcome of the event.

*Suspected adverse reaction* (SAR) means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, reasonable possibility means there is evidence to suggest a causal relationship between the intervention and the adverse event. Suspected adverse reaction implies a lesser degree of certainty than adverse reaction, which means any adverse event caused by a drug. Suspected adverse reactions are the subset of all AEs for which there is a reasonable possibility that the drug caused the event. Inherent in this definition, and in the requirement to report them is the need for the sponsor to evaluate the available evidence and make a judgment about the likelihood that the drug actually caused the adverse event.

An AE or SAR is considered unexpected if it is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed.

### 8.4 Definition of a Serious Adverse Event (SAE)

An AE or SAR is considered serious if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death;
- A life-threatening event (its occurrence places the subject at immediate risk of death);
- Inpatient hospitalization or prolongation of existing hospitalization;
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions; or
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment,

they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

A prescheduled, elective procedure, or a routinely scheduled treatment that requires hospitalization is NOT considered as to be an SAE; the study site must document all of the following:

- The prescheduled, or elective procedure, or routinely scheduled treatment was scheduled (or was on a waiting list to be scheduled) prior to obtaining the subject's consent to participate in the study.
- The condition requiring the prescheduled, or elective procedure, or routinely scheduled treatment was present before and did not worsen or progress, in the opinion of the Investigator, between the subject's consent to participate in the study and the time of the procedure or treatment. The prescheduled, or elective procedure, or routinely scheduled treatment is the sole reason for the intervention or hospital admission.

A distinction should be drawn between serious and severe events or reactions. A severe AE/SAR is a major event of its type. A severe AE/SAR does not necessarily need to be considered serious. For example, nausea which persists for several hours may be considered severe nausea, but would not be considered serious. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be considered serious.

If there is any doubt whether an AE qualifies as an SAE based on the definitions above, the Investigator should contact the Sponsor.

### 8.5 Grading of Adverse Events

Adverse events will be graded for intensity as mild, moderate, severe, life-threatening, or resulting in death as defined below. If applicable, the toxicity grade found in the Manual of Procedures should also be noted.

| Grade | Description |
|---|---|
| **Mild** | Symptom barely noticeable to the subject and does not influence his/her performance or functioning. |
| **Moderate** | Symptom of a sufficient severity to make the subject uncomfortable or hinder the performance of daily activities; treatment of symptom may be needed. |
| **Severe** | Symptom causes severe discomfort; treatment for symptom may be given and/or subject may be hospitalized. |
| **Life-threatening** | Occurrence of the event places the subject at immediate risk of death. |
| **Death** | Occurrence of the event results in the death of the subject. |

### 8.6 Relationship to the Investigational Product

The Investigator will document his/her opinion of the relationship of each AE to the vector administration procedure, investigational product, and study measures using the criteria outlined below. Relationship to the administration procedure should also be noted.

| Relationship | Description |
|---|---|
| **Unlikely to be related** | AEs which, after careful medical consideration at the time they are evaluated, are judged to be unrelated to the investigational material because:<br><br>a) AE does not follow a reasonable temporal sequence or known pattern of response after administration of the investigational material<br><br>b) AE could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. |
| **Possibly related** | AEs for which, after careful medical consideration at the time they are evaluated, a connection with the investigational material appears unlikely but cannot be ruled out with certainty, because at least one of the following criteria are met:<br><br>a) AE follows a reasonable temporal sequence or known pattern of response after administration of investigational material.<br><br>b) AE could not readily have been produced by subject's clinical state, toxic or environmental factors or other modes of therapy administered to the subject. |
| **Probably related** | AEs which, after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be related to the investigational material, because the following criteria are met:<br><br>a) AE cannot be reasonably explained by the known characteristics of the subject's clinical state or by environmental or toxic factors;<br><br>**and** at least one of the following is true:<br><br>b) AE follows a reasonable temporal sequence from administration of the investigational new drug and/or administration procedure;<br><br>c) AE follows a known pattern of response to the investigational new drug or administration procedure;<br><br>d) AE disappears or decreases on cessation or reduction in activity of the investigational new drug; or<br><br>e) AE reappears upon re-challenge or re-administration of the investigational new drug. |

Investigators are responsible for reporting SAEs and suspected unexpected serious adverse reactions (*i.e.,* SUSARs) to their respective Independent Reviewing Authorities (IRAs; may

also be known as Institutional Review Boards or Independent Ethics Committees) in accordance with local reporting requirements.

### 8.7 Other Reportable Events

Certain events that occur in the absence of an AE should be reported to Spark Therapeutics as other reportable events. The events will be recorded using the Other Reportable Information Form provided by the Sponsor. These include the following:

- Accidental exposure (someone other than the study subject was exposed to investigational product)
- Overdose (subject received more than the intended dose of investigational product)
- Other medication errors that potentially place subjects at a greater risk of harm than was previously known or recognized (*e.g.* investigational product was administered by an incorrect route).

### 8.8 Reporting Timeframes

The Investigators are responsible for notifying the Sponsor or designee of all AEs, other unanticipated problems related to research, and pregnancy occurrences and outcomes in accordance with the following timeline:

| Type of AE | Initial Notification (Phone, Email) | Written Report |
|---|---|---|
| All serious AEs, including serious SARs | 24 hours | The investigator (or delegate) must submit the SAE report forms (initial and follow-up) within 24 hours to the Sponsor/designee. Local telephone and fax number for reporting from each specific participating country is provided in the Investigator Study Manual. If the minimum requirements for reporting are known, the investigator should notify Spark immediately and not wait for additional information to fully document the event. Follow-up reports will be submitted by the investigator to Spark at appropriate intervals until the SAE has resolved or, in the case of permanent impairment, until it has stabilized. |
| Other unanticipated problems or reportable events related to research and pregnancy occurrences and outcomes | N/A | Within 5 business days |
| All other AEs | N/A | Summary of AEs reported at time of continuing review<br><br>As needed for sponsor reporting requirements (FDA & NIH/OBA annual reports and DSMB reports) or as specified in MOP |

All AEs must be entered in the appropriate section of the CRF. To improve the quality and consistency of AE data, Investigators should observe the following general guidelines:

- Standard English medical terminology should be used rather than colloquial expressions or abbreviations.

- Whenever possible, the AE should be evaluated and reported as a diagnosis rather than as individual signs or symptoms. If a definitive diagnosis is not possible, the individual signs and symptoms should be recorded.
- If an AE requires a surgical or diagnostic procedure, the illness leading to the procedure should be recorded as the AE, not the procedure itself.

For SAEs, the initial notification should include all available information requested on the SAE Form; the initial notification must be followed by written documentation, which includes all information including causality assessment from the Principle Investigator. The SAE Form will collect data surrounding the event, *e.g.,* the nature of the symptom(s), time of onset in relation to initiation of investigational product, duration, severity, and whether or not treatment was administered for the symptoms experienced. The Investigator's assessment of the probable cause of the event will also be included. A physician will examine subjects who experience an SAE or a medical emergency situation as soon as possible. The physician in attendance will do whatever is medically necessary for the safety and well-being of the subject. The subject will remain under observation as long as medically indicated in the opinion of the Investigator. Appropriate lab studies will be conducted until all parameters return to normal or are otherwise explained. The SAE will be followed until resolved or until medically stabilized. In a medical emergency requiring immediate attention, study site staff will provide appropriate medical care, according to current standards of care. The Investigator or his/her designee should contact the Sponsor or designee Medical Monitor. The Sponsor or designee will notify the appropriate federal regulatory authorities.

If an SAE has not resolved at the time of the initial report, a follow-up report including all relevant new or reassessed information (*e.g.,* concomitant medication, medical history) should be submitted to the Sponsor or designee. All SAEs should be followed until either resolved or stable. The Medical Monitor and the independent DSMB will review all AEs.

Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and Sponsor policy, and forwarded to Investigators as necessary. An Investigator who receives an investigator safety report describing an SAE or who receives other specific safety information (e.g., summary or listing of SAEs) from the Sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC if appropriate according to local regulatory reporting requirements.

### 8.9 Reporting of Partner Pregnancy Occurrences and Outcomes

Should a female partner of a subject become pregnant after the subject has been administered AAV2-hCHM, the subject will inform the Investigator as soon as possible. The Investigator will obtain the consent and authorization of medical information release from subject's female partner before following up with the subject and/or subject's female partner to obtain the eventual outcome of the pregnancy. Other information related to the pregnancy may be requested for safety reporting purposes. This information will be tracked by the Sponsor or its designee.

- If a partner pregnancy occurrence is reported, the Investigator should inform the Sponsor or its designee within 5 business days of learning of the pregnancy occurrence.
- The Investigator should follow up with the subject and/or subject's female partner at the expected time to obtain the pregnancy outcome.
- The Investigator should inform the Sponsor or its designee within 5 business days of learning of the pregnancy outcome.
- Abnormal pregnancy outcomes including but not limited to spontaneous abortion, fetal death, stillbirth, congenital anomalies, and ectopic pregnancy are considered SAEs.
- Pregnancy information for female partners of subjects if the female partners were already known to be pregnant at the time of AAV2-hCHM administrations should be collected as medical history.

# 9 STUDY ADMINISTRATION

## 9.1 Dose Group Assignment Methods

This is an open-label, non-randomized, non-blinded inter-group dose escalation safety and tolerability study of AAV2-hCHM, administered unilaterally by single subretinal vector delivery. Barring toxicity, subjects will be sequentially entered to Dose Group 1, then Dose Group 2.

## 9.2 Data Collection and Management

Complete and accurate records are essential for the performance of a clinical trial and accurate interpretation of its results. These records will be maintained in a secure area with limited access, but will be available and attainable for audits as required by federal, local, or institutional regulatory agencies. All trial registration documents will be obtained and pass a compliance review before shipment of investigational drug to a study site will be authorized by the Sponsor.

The investigative team is responsible for maintaining a comprehensive and centralized filing system of all relevant study documentation including:

- Case report forms
- Subject source document files
- Monitoring logs
- Registration documents (including Clinical Protocol and Investigator's Brochure, curriculum vitae of participating Investigators, IRB correspondence, signed 1572)
- Drug accountability records

Data will be entered from subject source documents to Case Report Forms that are specially designed for this study. The Investigators are responsible for accurate completion of these forms for all subjects who receive AAV2-hCHM in the study. These forms are an integral part of the study and must be completed.

All data and records generated during this study will be kept confidential in accordance with institutional policies and Health Insurance Portability and Accountability Act (HIPAA) on subject privacy. Investigators and other site personnel will not use the data and records for any purpose other than conducting the study. Study subjects should be identified on Case Report Forms by study number, not by name or initials, to protect subject privacy. The Investigators will keep a separate log of subject names and addresses in a locked cabinet and/or on a separate computer as a password protected file. However, in compliance with FDA Good Clinical Practice regulations, the study monitor may review that portion of the subject's medical record that is directly related to the study.

All trial Essential Documents will be retained in the study site files for a minimum of two years after the date of approval for commercial license of a biologic, device, or drug, last approval of a marketing application in an ICH region, until there are no pending or contemplated marketing applications in an ICH region, or until at least two years have elapsed since the formal discontinuation of clinical development of the investigational material.

## 9.3 Subject Confidentiality

All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy, and Investigators and other site personnel will not use such data and records for any purpose other than conducting the study. The safeguards to maintain subject confidentiality include training all study personnel in HIPAA guidelines for study privacy and security. Subject specimens will be coded according to the designation provided by the master subject list.

## 9.4 Regulatory and Ethical Considerations

### 9.4.1 Data and Safety Monitoring Plan

The Sponsor will follow a Data and Safety Monitoring Plan for this study. The study sites will be monitored by an independent contract research organization for compliance with FDA regulations and ICH Good Clinical Practice by qualified and trained personnel. In addition, FDA may audit the clinical study sites and records for regulatory compliance. The Principal Investigators and staff are expected to cooperate and provide all relevant study documentation upon request for review by the auditors. All Investigators, including those responsible for administration and/or clinical follow-up evaluations, will be instructed in Good Clinical Practice (GCP) Guidelines and clinical protocol requirements.

An independent DSMB will be established. This board will provide oversight and monitoring of the conduct of the trial to ensure the safety of participants and the validity and integrity of the data. Members of the DSMB will include clinicians, scientists, and public representatives. These individuals will be selected on the basis of their experience, knowledge of clinical trial methodology, and absence of conflicts of interest. The DSMB will review and approve the research protocols and detailed plans for data and safety monitoring. It will establish specific guidelines for monitoring for safety and review interim outcome data for safety and efficacy. The DSMB will also review trial performance information such as subject recruitment and retention, clinical site performance, and proposals for ancillary studies. It will also review published reports of related studies to determine whether this study needs to be changed or terminated. DSMB meetings, in person or by teleconference, will occur no less frequently than

annually while subjects are actively enrolling in the study. Following each DSMB meeting, the DSMB will provide the study leaders with written recommendations related to continuing, changing, or terminating the trial.

Prior to implementation, any amendments to the protocol that have subject safety implications must be approved by the IRB and DSMB, and submitted to the IND on file with FDA. Measures that are critical for subject safety may be implemented simultaneously with reporting to the IRB, DSMB, and FDA. Any manuscript, abstract, or presentation will be available to all the Investigators involved in this study, as well as the DSMB as described above, for review prior to submission.

### 9.4.2 Risk Assessment

Clinical experience of gene therapy for the eye has been based on AAV gene delivery for *RPE65* mutations, using the AAV2-hRPE65v2 vector. Possible adverse events for subjects enrolled in this study include: risks associated with the administration procedure (subretinal delivery) and risks associated with the investigational material (AAV2-hCHM).

Risks associated with the administration procedure (subretinal delivery)

- Cataract including cataract subcapsular and cataract nuclear;
- Conjunctival hemorrhage;
- Eye pain;
- Diplopia;
- Eye irritation;
- Posterior capsule opacification;
- suture related complication;
- Intraocular pressure increased;
- Corneal disorder;
- Corneal epithelium defect;
- Dellen (localized dehydration of the cornea);
- Eye pruritis;
- Macular hole;
- Macular cyst;
- Visual acuity reduced;
- Headache.

Additional potential risk, not listed above, that have been associated with the administration procedure and investigational material in the AAV2-hRPE65v2 studies are:

- Retinal tear and retinal detachment
- Ocular inflammation or infection
- Macular disorders
- Vitreous opacities

A small scar at the retinotomy site is expected, as part of the healing process post-surgery. The scar results from penetration of the retina with the subretinal injection cannula and the subsequent apposition of the cannula against the underlying RPE. Since the postnatal retina is

terminally differentiated, cells do not divide to replace those that are damaged by retinal penetration. Instead, glial cells fill in the neural retinal penetration site and RPE cells directly underlying this site can migrate towards the retina. The net result of these changes is that the boundaries of the different tissue layers are maintained. The retinotomy site will result in a blind spot smaller than the physiologic blind spot resulting from the presence of the optic disc and, indeed is not detectable with standard visual field testing; it will not involve the central portion of the macula. It is highly unlikely that the subject will be aware of this blind spot or that it will have a negative impact on visual function as neither global retinal function nor visual acuity (function of the macula/fovea) is affected by the retinotomy scar. It would require sophisticated visual field analysis in a person with good vision to detect a decrease in retinal function after retinotomy in this point location. The retinotomy scar is not expected to cause diminished vision in subjects participating in this trial.

Other expected AE would be those related to surgery, including post-operative inflammation such as corneal edema or abrasion, mild anterior chamber reaction (cell and flare), mild lens opacity ("feathering"), change in eye pressure, diplopia, mild choroidal edema, and retinal edema or hemorrhage at the site of injection. The symptoms, if any, would be blurred vision, photophobia, lacrimation and iritis-type eye discomfort. These are treatable by cycloplegia (atropine drops), anti-inflammatory medication with medicine administered locally (eye drops or injection) or systemically (steroidal or non-steroidal).

In the clinical and non-clinical studies done to date, more serious possible AE were not observed, including corneal decompensation with opacification, glaucoma in the treated eye, iris atrophy with synechiae, persistent vitreous opacification, persistent retinal detachment (exudative or rhegmatogenous), retinal membrane with proliferative vitreoretinopathy, optic atrophy, cystoid macular edema (retinal swelling), and hypotony with eventual phthisis bulbi (ocular atrophy). If these events occur, some would be anticipated to resolve without treatment, while others would require treatment. Treatment for corneal de-compensation would involve corneal transplant, cataract would require extraction, vitrectomy would be used to treat vitreous opacities, retinal reattachment procedures would be used to treat non-resolving retinal detachment, filtration surgery or medication could be used to treat glaucoma, infection treated with antibiotics, macular edema would require treatment with anti-inflammatory agents. The most serious AE would be intractable glaucoma or phthisis bulbi, which could lead to a blind, painful eye. Those conditions can be treated with a peri-ocular injection of alcohol or Thorazine to treat the pain or eventual enucleation, if absolutely necessary. The worst possible morbidity would be complete loss of vision.

General Anesthesia Risks

Serious but rare adverse events from general anesthesia and surgery in otherwise healthy individuals include seizure, coma and death.

Risks Based on Non-Clinical Studies

A toxicology study in non-human primates evaluating two doses of GMP-comparable AAV2-hCHM vector ($3x10^{11}$ and $7.5x10^{11}$ vg/eye) showed that the higher dose, which is 15 times greater than the low dose to be evaluated in this study, resulted in mild and focal loss of photoreceptors. These changes were observed 3 months post-injection, but not at the earlier (3

week) time point. Ocular histopathology at 3 weeks in both vehicle and AAV-injected eyes showed the predicted pathological changes due to the surgical manipulations; these included mild inflammatory lesions and local trauma to the RPE cells at the site of injection. Inflammation was present in many of the eyes, and there was a trend toward increasing inflammation related to the higher dose of AAV2-hCHM and the longer time to sacrifice of animals. On the numeric inflammatory scale used to score the changes in these eyes (from 0-4, with 4 being severe inflammation), there were no eyes in the study with an inflammatory score greater than 1. This indicates that in the eyes with the most prominent inflammatory changes, the inflammation was mild.

It is not clear what the mild focal loss of photoreceptors observed in animals injected with the high dose of AAV2-hCHM and sacrificed at day 91 was due to. The possibilities include: complications of the subretinal injection, the AAV2 capsid, or expression of human REP-1 in cynomolgus monkeys. Since higher doses of AAV2-hRPE65 did not lead to photoreceptor loss when injected into the eyes of this same species of monkeys, this finding is not likely due to the AAV2 capsid. However, it is possible that it could be due to expression of a foreign transgene, as there are 18 amino acid differences between the human and Cynomolgus REP-1 proteins, while there is only a single amino acid difference between the human and Cynomolgus hRPE65 proteins. Thus, it is more likely that the hREP-1 protein will be viewed as a foreign antigen than hRPE65.

Potential Risks of AAV2-hCHM

Recombinant AAV is engineered from a non-pathogenic parvovirus that naturally infects humans, most likely through the respiratory tract during childhood. Prior exposure to wild-type AAV may contribute to the immune response and it is possible that prior AAV vector administration could contribute as well. While these scenarios pose the risk of immune responses that potentially decrease the effectiveness of vector administration, and even though immune reactivity has been demonstrated in the AAV trials for hemophilia, consequential immune reactivity has not so far been seen in this trial. Neither significant immune responses to either the capsid or transgene have been detected. While sequential injection of an AAV vector has the potential for complicating immune responses, immune responses have not been significant, and have not resulted in untoward effects on efficacy in this study nor in the phase 1 trial of AAV2-hRPE65v2, or in the follow-on trial (Maguire *et al.*, 2008; Maguire *et al.*, 2009; Bennett *et al.*, 2012).

An immune response is not expected based on the relatively small dose of vector to be administered in this study, compared similarly to the dose of AAV2-hRPE65v2 used for inherited retinal degeneration due to autosomal-recessive *RPE65* gene mutations. When compared to the dose of AAV2-hFIX (IND #9398, inactive) that was under study for hemophilia B, the maximum total dose to be administered to subjects in this eye study is approximately 250 times less than the total dose at which the cell-mediated responses (described above) in the liver trial occurred. Accordingly, the dose of AAV2-hCHM used for this study of choroideremia compares favorably to the dose of AAV2-hRPE65v2 used for the RPE65 trials, and not expected to result in immune responses that prevent efficacy or successful injection into the contralateral eye during follow-on studies.

One hypothetical adverse event would be the unexpected effects of the transgene on function of

the central nervous system (CNS) due to transduction of ganglion cells (which send their axons to the brain via the optic nerve). This event is considered unlikely; however, if there was evidence of CNS dysfunction associated with retinal transgene expression, laser ablation or a complete retinectomy could be performed. This would destroy the ganglion cell axons and the brain would no longer be exposed to the transgene product.

### 9.4.3 Potential Benefits of Trial Participation

This is a Phase 1/2 safety, tolerability and dose escalation study of the subretinal administration of the AAV2-hCHM vector to subjects with choroideremia. A secondary objective is to measure efficacy and immune responses against AAV capsid and the transgene product. It is not known if the doses used in this study will be sufficient to either arrest retinal degeneration or improve vision in the administered subjects. Similar doses of the vector used in the RPE65 trials demonstrated safety and efficacy. Thus, from previous clinical experience it is suspected that the doses of vector proposed here are likely to be safe.

The starting dose for the trial is $5x10^{10}$ vg per eye, which is roughly equivalent to the middle dose previously used in the RPE65 Phase 1 trial of AAV2-hRPE65v2, and which resulted in efficacy in the 6 subjects who received this dose of vector. Since the current study is employing the same serotype but a different transgene, and the non-clinical data suggests that this may provide efficacy (Section 1.4), it seems reasonable to start within the dose range used in the RPE65 trial. Dose Group 2 ($1x10^{11}$ vg per eye) is also within the dose range established in the RPE65 studies and is comparable to the high dose used for the RPE65 Phase 1 trial. Further, the Sponsor anticipates lack of toxicity at these doses, based on the non-clinical data and pharmacology/toxicology data in non-human primates (Section 1.4).

Based on the earlier data from clinical trials conducted under FDA IND # 13408, and from other trials of inherited retinal degeneration due to autosomal-recessive *RPE65* gene mutations, as well as the non-clinical data that are described in section 1.4, it is possible that the proposed starting Dose 1 of $5x10^{10}$ vg per eye has the potential to yield stabilization or improvement in vision in affected subjects.

### 9.4.4 Risk-Benefit Assessment

There are a number of risks associated with the proposed research study. On the basis of the available non-clinical and clinical data to date, the Sponsor believes the potential risks are balanced by the potential to gain knowledge about the disease, and a potential for direct benefit to the subjects. The anticipated benefit:risk ratio is at least as favorable as the alternative approaches, as there is no FDA approved treatment for choroideremia. In this research context, there may be potential risks that are unexpected or unknown.

## 9.5 Recruitment Strategy

Potential subjects will be recruited from the clinics of investigative team members, from ophthalmology treatment centers, as well as subjects referred from health-care providers and self-referred subjects via materials posted in clinicaltrials.gov. After description of the clinical trial design to the subject, using the study consent and other approved recruitment materials as educational tools, if the subject wishes to pursue the gene therapy study, the consenting process will ensue, and if the subject agrees to study participation, the study-mandated

Screening/Baseline tests will be initiated. It is expected that there are sufficient subjects who will meet eligibility criteria for study entry.

### 9.6 Informed Consent and HIPAA Authorization

Following study approval by the governing regulatory groups, potential subjects who have met initial clinical criteria are approached by a member of the investigative team to determine the level of interest for study enrollment. Potential subjects will generally be approached in person, called on the telephone, or emailed to initiate the consenting process. The study's informed consent documents are used as an "educational tool" for the subjects and subjects' female partners to read, and ask questions of the Investigators. The content of the informed consent documents follows federal regulatory requirements. The informed consent process will take place in a private setting, for example in a clinic or hospital room, or in a doctor's office. Steps will be taken to avoid coercion or undue inducements; the subject or subject's female partner will be given as much time as needed to make a decision about study enrollment and/or release of medical information. The consent will be signed only after the subject and/or subject's female partner have had all questions answered to their satisfaction. The Investigators will ensure that subjects understand that participation in the study is voluntary and subjects and subjects' female partners understand that authorization of medical information release is voluntary. Subjects will have the option to withdraw from study participation and authorization of medical information release can be terminated when requested. Both the subject/female partner and the person obtaining consent (a designated member of the investigative team) will sign and date the consent document, and a copy will be given to the subject/female partner. The study or data collection can be initiated only after consent signing. Non-English speaking subjects are not expected for enrollment; however, if a non-English speaking subject is considered for enrollment or a non-English speaking female partner is pregnant, a certified translated and IRB-approved consent with independent interpreter would be implemented for the consenting process.

### 9.7 Financial Considerations of Study Participation

There will be no payment (compensation) or gifts to subjects or families. The Sponsor will arrange for or provide reimbursement for travel, parking, hotels and meals to the subject and one companion, if necessary. The costs of the long-term follow-up will be borne by the Sponsor.

## 10 PUBLICATION

Upon completion of the study, the Investigators may publish the results in recognized scientific journals subject to the provisions of the Clinical Trial Agreement (CTA). Unless otherwise specified in the CTA, the following process shall occur:

The institution and Investigators shall not publish or present data from an individual study center until the complete multicenter study has been presented in full or for two years after the termination of the multicenter study, whichever occurs first, or without written Sponsor approval. Subsequent publications must refer to the multicenter findings. Thereafter, if the Investigators expects to participate in the publication of data generated from this site, the institutions and Investigators shall submit reports, abstracts, manuscripts, and/or other

presentation materials to the Sponsor for review before submission for publication or presentation. The Sponsor shall have 60 days to respond with any requested revisions, including, without limitation, the deletion of confidential information. The Principal Investigators shall act in good faith upon requested revisions, except that the Principal Investigators shall delete any confidential information from such proposed publication.

Any manuscript, abstract, or presentation will be available to all the Investigators involved in this study and the DSMB for review prior to submission. Information about the conduct and progress of the study is not to be discussed beyond the study teams and Sponsor representatives, including contract research organization and DSMB members, necessary to conduct the study. Study data are confidential and not to be disclosed without written Sponsor approval.

## 11 REFERENCES


Acland, G.M., Aguirre, G.D., Ray, J., Zhang, Q., Aleman, T.S., Cideciyan, A.V.,…Bennett, J. (2001). Gene therapy restores vision in a canine model of childhood blindness. *Nature Genetics*, *28*, 92-95. doi:10.1038/ng0501-92

Acland, G.M., Aguirre, G.D., Bennett, J., Aleman, T.S., Cideciyan, A.V., Bennicelli, J.,…Jacobson, S.G. (2005). Long-term restoration of rod and cone vision by single dose rAAV-mediated gene transfer to the retina in a canine model of childhood blindness. *Molecular Therapy: The Journal of the American Society of Gene Therapy*, *12*(6), 1072-82. doi:10.1016/j.ymthe.2005.08.008

Alexandrov, K., Horiuchi, H., Steele-Mortimer, O., Seabra, M.C., & Zerial, M. (1994). Rab escort protein-1 is a multifunctional protein that accompanies newly prenylated rab proteins to their target membranes. *The EMBO Journal, 13*(22), 5262-5273.

Amado, D., Mingozzi, F., Hui, D., Bennicelli, J.L., Wei, Z., Chen, Y.,…Bennett, J. (2010). Safety and efficacy of subretinal readministration of a viral vector in large animals to treat congenital blindness. *Science Translational Medicine*, *2*(21), 21ra16. doi:10.1126/scitranslmed.3000659

Anand, V., Duffy, B., Yang, Z., Dejneka, N.S., Maguire, A.M., & Bennett, J. (2002). A deviant immune response to viral proteins and transgene product is generated on subretinal administration of adenovirus and adeno-associated virus. *Molecular Therapy: The Journal of the American Society of Gene Therapy*, *5*(2), 125-132. doi: 10.1006/mthe.2002.0525

Bainbridge, J.W.B., Smith, A.J., Barker, S.S., Robbie, S., Henderson, R., Balaggan, K.,…Ali, R.R. (2008). Effect of gene therapy on visual function in leber's congenital amaurosis. *The New England Journal of Medicine*, *358*(21), 2231-2239. doi: 10.1056/NEJMoa0802268

Basiji, D.A., Ortyn, W.E., Liang, L., Venkatachalam, V., & Morrissey, P. (2007). Cellular image analysis and imaging by flow cytometry, *Clinics in Laboratory Medicine*, *27*(3), 653-670. doi: 10.1016/j.cll.2007.05.008

Bennett, J., Duan, D., Engelhardt, J.F., & Maguire, A.M. (1997). Real-time, noninvasive in vivo assessment of adeno-associated virus-mediated retinal transduction. *Investigative Ophthalmology & Visual Science*, *38*(12), 2857-2863.

Bennett, J., Maguire, A.M., Cideciyan, A.V., Schnell, M., Glover, E., Anand, V.,…Jacobson, S.G. (1999). Stable transgene expression in rod photoreceptors after recombinant adeno-associated virus-mediated gene transfer to monkey retina. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(17), 9920-9925. doi: 10.1073/pnas.96.17.9920

Bennett, J. (2003). Immune response following intraocular delivery of recombinant viral vectors. *Gene Therapy, 10*(11), 977-982. doi:10.1038/sj.gt.3302030

Bennett, J., Ashtari, M., Wellman, J., Marshall, K.A., Cyckowski, L.L., Chung, D.C.,… Maguire, A.M. (2012). AAV2 gene therapy readministration in three adults with congenital blindness. *Science Translational Medicine*, *4*(120), 120ra15. doi: 10.1126/scitranslmed.3002865

Bennett, J., Wellman, J., Marshall, K.A., McCague, S., Ashtari, M., DiStefano-Pappas, J.,… Maguire, A.M. (2016). Safety and durability of effect of contralateral-eye administration of AAV2 gene therapy in patients with childhood-onset blindness caused by RPE65 mutations: A follow-on phase 1 trial. *Lancet (London, England), 388*(10045), 661-72. doi: 10.1016/S0140-6736(16)30371-3

Bennicelli, J., Wright, J.F., Komaromy, A., Jacobs, J.B., Hauck, B., Zelenaia, O.,… Bennett, J. (2008). Reversal of blindness in animal models of leber congenital amaurosis using optimized AAV2-mediated gene transfer. *Molecular Therapy: The Journal of the American Society of Gene Therapy*, *16*(3), 458-465. doi: 10.1038/sj mt.6300389

Buchlis, G., Podsakoff, G.M., Radu, A., Hawk, S.M., Flake, A.W., Mingozzi, F.,… High, K.A. (2012). Factor IX expression in skeletal muscle of a severe hemophilia b patient 10 years after AAV-mediated gene transfer. *Blood*, *119*(13), 3038-3041. doi: 10.1182/blood-2011-09-382317

Chow, D.R., Williams, G.A., Trese, M.T., Margherio, R.R., Ruby, A.J., & Ferrone, P.J. (1999). Successful closure of traumatic macular holes. *Retina*, *19*(5), 405-409.

Cideciyan, A.V., Jacobson, S.G., Beltran, W.A., Sumaroka, A., Swider, M., Simone, I.,… Aguirre, G.D. (2013). Human retinal gene therapy for leber congenital amaurosis shows advancing retinal degeneration despite enduring visual improvement. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(6), E517-E525. doi: 10.1073/pnas.1218933110

Couto, L. (2016). AAV gene therapy for choroideremia: Dose determination analyses. *The Association for Research in Vision and Ophthalmology Meeting.*

Cremers, F.P., Armstrong, S.A., Brown, M.S., & Goldstein, J.L. (1994). REP-2, a rab escort protein encoded by the choroideremia-like gene. *The Journal of Biological Chemistry*, *269*(3), 2111-7.

Dejneka, N.S., Surace, E.M., Aleman, T.S., Cideciyan, A.V., Lyubarsky, A., Savchenko, A.,… Bennett, J. (2004). In utero gene therapy rescues vision in a murine model of congenital blindness. *Molecular Therapy: The Journal of the American Society of Gene Therapy*, *9*(2), 182-188. doi: 10.1016/j.ymthe.2003.11.013

Donsante, A., Miller, D.G., Li, Y., Vogler, C., Brunt, E.M., Russell, D.W.,… Sands, M.S. (2007). AAV vector integration sites in mouse hepatocellular carcinoma. *Science*, 317-477. doi: 10.1126/science.1142658

Edwards, T.L., Jolly, J.K., Groppe, M., Barnard, A.R., Cottriall, C.L., Tolmachova, T.,… MacLaren, R.E. (2016). Visual acuity after retinal gene therapy for choroideremia. *The New England Journal of Medicine*, *374*(20), 1996–1998. doi: 10.1056/NEJMc1509501

Fighting Blindness Canada. (2012, February 15). Foundation Fighting Eye disease fact sheet: Choroideremia. Retrieved from http://www.ffb.ca/eye_conditions/choroideremia html?PHPSESSID=f7e881c77bc902024ee61fc00c2f18e5

Genetics Home Reference. (2008, July). Choroideremia. Retrieved from http://ghr nlm.nih.gov/condition/choroideremia

Graham, F.L., Smiley, J., Russell, W.C., & Nairn, R. (1977). Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *The Journal of General Virology*, *36*(1), 59-72. doi: 10.1099/0022-1317-36-1-59

Hauswirth, W.W., Aleman, T.S., Kaushal, S., Cideciyan, A.V., Schwartz, S.B., Wang, L.,… Jacobson, S.G. (2008). Treatment of leber congenital amaurosis due to RPE65 mutations by ocular subretinal injection of adeno-associated virus gene vector: Short-term results of a phase I trial. *Human Gene Therapy*, *19*(10), 979-90. doi: 10.1089/hum.2008.107

Ignatova, I. (2016). AAV gene therapy for choroideremia: Dose determination analyses. *American Society of Gene & Cell Therapy Meeting.*

Jacobs, J.B., Dell-Osso, L.F., Hertle, R.W., Acland, G.M., & Bennett, J. (2006). Eye movement recordings as an effectiveness indicator of gene therapy in RPE65-deficient canines: Implications for the ocular motility system. *Investigative Ophthalmology & Visual Science*, *47*(7), 2865-2875. doi: 10.1167/iovs.05-1233

Jacobson, S.G., Aleman, T.S., Cideciyan, A.V., Sumaroka, A., Schwartz, S.B., Windsor, E.A.M,… Bennett, J. (2005). Identifying photoreceptors in blind eyes caused by RPE65 mutations: Prerequisite for human gene therapy success. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(17), 6177-6182. doi: 10.1073/pnas.0500646102

Jacobson, S.G., Cideciyan, A.V., Sumaroka, A., Aleman, T.S., Schwartz, S.B., Windsor, E.A.,… MacDonald, I.M. (2006). Remodeling of the human retina in choroideremia: Rab escort protein 1 (REP-1) mutations. *Investigative Ophthalmology & Visual Science*, *47*(9), 4113-20. doi: 10.1167/iovs.06-0424

Jacobson, S.G., Acland, G.M., Aguirre, G.D., Aleman, T.S., Schwartz, S.B., Cideciyan, A.V.,… Hauswirth, W.W. (2006). Safety of recombinant adeno-associated virus type 2-RPE65 vector delivered by ocular subretinal injection. *Molecular Therapy: The Journal of the American Society of Gene Therapy*, *13*(6), 1074-84. doi: 10.1016/j.ymthe.2006.03.005

Jacobson, S.G., Boye, S.L., Aleman, T.S., Conlon, T.J., Zeiss, C.J., Roman, A.J.,…Hauswirth, W.W. (2006). Safety in nonhuman primates of ocular AAV2-RPE65, a candidate treatment for blindness in leber congenital amaurosis. *Human Gene Therapy*, *17*(8), 845-858. doi: 10.1089/hum.2006.17.845

Jacobson, S.G., Cideciyan, A.V., Ratnakaram, R., Heon, E., Schwartz, S.B., Roman, A.J.,…Hauswirth, W.W. (2012). Gene therapy for leber congenital amaurosis caused by RPE65 mutations: Safety and efficacy in 15 children and adults followed up to 3 years. *Archives of Ophthalmology (Chicago, III. : 1960) 130*(1), 9-24. doi: 10.1001/archophthalmol.2011.298

Jiang, H., Pierce, G., Ozelo, M., de Paula, E., Vargas, J., Smith, P.,… Arruda, V. (2006). Evidence of multiyear factor IX expression by AAV-mediated gene transfer to skeletal muscle in an individual with severe hemophilia b. *Molecular Therapy: The Journal of the American Society of Gene Therapy*, *14*(3), 452-455. doi: 10.1016/j.ymthe.2006.05.004

Kozak, M. (1997). Recognition of AUG and alternative initiator codons is augmented by G in position F4 but is not generally affected by the nucleotides in positions F5 and F6. *The EMBO Journal*, *16*(9), 2482–2492. doi: 10.1093/emboj/16.9.2482

Li, H., Malani, N., Hamilton, S.R., Schlachterman, A., Bussadori, G., Edmonson, S.E.,… High, K.A. (2011). Assessing the potential for AAV vector genotoxicity in a murine model. *Blood*, *117*(24), 3311-3319. doi: 10.1182/blood-2010-08-302729

MacDonald, I.M., Russell, L., Chan, C.C. (2009). Choroideremia: New findings from ocular pathology and review of recent literature. *Survey of Ophthalmology*, *54*(3), 401-407. doi: 10.1016/j.survophthal.2009.02.008

MacLaren, R. (2012). Treatment of choroideremia in humans by gene replacement. *The Association for Research in Vision and Ophthalmology Meeting.*

MacLaren, R.E., Groppe, M., Barnard, A.R., Cottriall, C.L., Tolmachova, T., Seymour, L.,… Seabra, M.C. (2014). Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial. *Lancet (London, England), 383*(9923), 1129-1137. doi: 10.1016/S0140-6736(13)62117-0

Maguire, A.M., Simonelli, F., Pierce, E.A., Pugh, E.N., Mingozzi, F., Bennicelli, J,… Bennett, J. (2008). Safety and efficacy of gene transfer for leber congenital amaurosis. *The New England Journal of Medicine*, *358*(21), 2240-2248. doi: 10.1056/NEJMoa0802315

Maguire, A.M., High, K.A., Auricchio, A., Wright , J.F., Pierce, E.A., Testa, F.,… Bennett, J. (2009). Age-dependent effects of RPE65 gene therapy for leber's congenital amaurosis: A phase 1 dose-escalation trial. *Lancet (London, England)*, *374*(9701), 1597-1605. doi: 10.1016/S0140-6736(09)61836-5

Manno, C.S., Chew, A.J., Hutchison, S., Larson, P.J., Herzog, R.W., Arruda, V.R.,… Glader, B. (2003). AAV-mediated factor IX gene transfer to skeletal muscle in patients with severe hemophilia b. *Blood*, *101*(8), 2963-72. doi: 10.1182/blood-2002-10-3296

McCarty, D.M., Young, S.M., & Samulski, R.J. (2004). Integration of adeno-associated virus (AAV) and recombinant AAV vectors. *Annual Review of Genetics*, *38*, 819-45. doi: 10.1146/annurev.genet.37.110801.143717

McGrath, K.E., Bushnell, T.P., & Palis, J. (2008). Multispectral imaging of hematopoietic cells: Where flow meets morphology. *Journal of Immunological Methods*, *336*(2), 91-97. doi: 10.1016/j.jim.2008.04.012

Mingozzi, F., & High, K.A. (2011). Therapeutic in vivo gene transfer for genetic disease using AAV: Progress and challenges. *Nature Reviews Genetics*, *12*(5), 341-55. doi: 10.1038/nrg2988

Mura, M., Sereda, C., Jablonski, M.M., MacDonald, I.M., & Iannaccone, A. (2007). Clinical and functional findings in choroideremia due to complete deletion of the *CHM* gene. *Archives of Ophthalmology (Chicago, III. : 1960)*, *125*(8), 1107-13. doi: 10.1001/archopht.125.8.1107

Nussbaum, R.L., Lewis, R.A., Lesko, J.G., & Ferrell, R. (1985). Choroideremia is linked to the restriction fragment length polymorphism DXYSJ at XQ13-21. *American Journal of Human Genetics, 37*(3), 473-481.

Orphanet. (n.d.) Choroideremia, OMIM 303100. Retrieved from http://www.orpha.net/consor/cgi-bin/Disease_Search.php?lng=EN&data_id=921&Disease_Disease_Search_diseaseGroup=Choroideremia&Disease_Disease_Search_diseaseType=Pat&Disease(s)/group%20of%20diseases=Choroideremia&title=Choroideremia&search=Disease_Search_Simple

Roberts, M.F., Fishman, G.A., Roberts, D.K., Heckenlively, J.R., Weleber, R.G., & Anderson, RJ. (2002). Retrospective, longitudinal, cross sectional study of visual acuity impairment in choroideraemia. *The British Journal of Ophthalmology*, *86*(6), 658-62. doi: 10.1136/bjo.86.6.658

Rosenberg, T., & Schwartz, M. (1994). Age differences of visual field impairment and mutation spectrum in Danish choroideremia patients. *Acta Ophthalmology*, *72*(6), 678-82. doi: 10.1111/j.1755-3768.1994.tb04679.x

Russell, S., Bennett, J., Wellman, J.A., Chung, D.C., Yu, Z.F., Tillman, A.,… Maguire, A.M. (2017). Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: A randomised, controlled, open-label, phase 3 trial. *Lancet (London, England)*, *390*(10097), 848-860. doi: 10.1016/S0140-6736(17)31868-8.

Samulski, R.J., Berns, K.I., Tan, M., & Muzyczka, N. (1982). Cloning of adeno-associated virus into pBR322: Rescue of intact virus from the recombinant plasmid in human cells. *Proceedings of the National Academy of Sciences of the United States of America*, *79*(6), 2077-2081. doi: 10.1073/pnas.79.6.2077

Samulski, R. J., Chang, L.S., & Shenk, T. (1987). A recombinant plasmid from which an infectious adeno-associated virus genome can be excised in vitro and its use to study viral replication. *Journal of Virology*, *61*(10), 3096-3101.

Seabra, M.C., Brown, M.S., & Goldstein, J.L. (1993). Retinal degeneration in choroideremia: Deficiency of rab geranylgeranyl transferase. *Science (New York, N.Y.)*, *259*(5093), 377-381. doi: 10.1126/science.8380507

Seabra, M.C., Ho, Y.K., & Anant, J.S. (1995). Deficient geranylgeranylation of Ram/Rab27 in choroideremia. *The Journal of Biological Chemistry*, *270*(41), 24420-24427. doi: 10.1074/jbc.270.41.24420

Simonelli, F., Maguire, A.M., Testa, F., Pierce, E.A., Mingozzi, F., Bennicelli, JL,… Auricchio, A. (2010). Gene therapy for leber's congenital amaurosis is safe and effective through 1.5 years after vector administration. *Molecular Therapy: The Journal of the American Society of Gene Therapy*, *18*(3), 643-650. doi: 10.1038/mt.2009.277

Streilein, J.W., Niederkorn, J.Y., & Shadduck, J.A. (1980). Systemic immune unresponsiveness induced in adult mice by anterior chamber presentation of minor histocompatibility antigens. *Journal of Experimental Medicine*, *152*(4), 1121-1125. doi: 10.1084/jem.152.4.1121

Tolmachova, T., Anders, R., Abrink, M., Bugeon, L., Dallman, M.J., Futter, C.E.,… Seabra, M.C. (2006). Independent degeneration of photoreceptors and retinal pigment epithelium in conditional knockout mouse models of choroideremia. *The Journal of Clinical Investigation*, *116*(2), 386-394. doi: 10.1172/JCI26617

Tolmachova, T., Tolmachov, O.E., Wavre-Shapton, S.T., Tracey-White, D., Futter, C.E., & Seabra, M.C. (2012). CHM/REP1 cDNA delivery by lentiviral vectors provides functional expression of the transgene in the retinal pigment epithelium of choroideremia mice. *The Journal of Gene Medicine*, *14*(3), 158-168. doi: 10.1002/jgm.1652

Vandenberghe, L.H., Bell, P., Maguire, A.M., Cearley, C.N., Xiao, R., Calcedo, R.,… Bennett, J. (2011). Dosage thresholds for AAV2 and AAV8 photoreceptor gene therapy in monkey. *Science Translational Medicine*, *3*(88), 88ra54. doi: 10.1126/scitranslmed.3002103

van den Hurk, J.A., Hendriks, W., van de Pol, D.J., Oerlemans, F., Jaissle, G., Rüther, K.,… Cremers, F. (1997). Mouse choroideremia gene mutation causes photoreceptor cell degeneration and is not transmitted through the female germline. *Human Molecular Genetics*, *6*(6), 851-858. doi: 10.1093/hmg/6.6.851

Vasireddy, V, Mill, J.A., Gaddameedi, R., Basner-Tschakarjan, E., Kohnke, M., Black, A.D.,… Bennett J. (2013). AAV-mediated gene therapy for choroideremia: Preclinical studies in personalized models. *PLOS ONE*, *8*(5), e61396. doi: 10.1371/journal.pone.0061396

Weckerle, P. (2009). Choroideremia. In *Encyclopedia of Molecular Mechanisms of Disease*. Retrieved from http://books.google.com/books?id=DdBJ6jCf8KgC&pg=PA349&dq=Encyclopedia+of+Molecular+Mechanisms+of+Disease,+choroideremia;&hl=en&sa=X&ei=sEvKUfrdNYeG0QHQsIH4Bg&ved=0CC8Q6AEwAA#v=onepage&q=Encyclopedia%20of%20Molecular%20Mechanisms%20of%20Disease%2C%20choroideremia%3B&f=false

Wright, J.F. (2008). Manufacturing and characterizing AAV-based vectors for use in clinical studies. *Gene Therapy*, *15*(11), 840-848. doi: 10.1038/gt.2008.65

Wright, J.F., Wellman, J., & High, K.A. (2010). Manufacturing and regulatory strategies for clinical AAV2-hRPE65. 2010. *Current Gene Therapy*, *10*(5), 341-349. doi: 10.2174/156652310793180715

Wright, J.F, & Zelenaia, O. (2011). Vector characterization methods for quality control testing of recombinant adeno-associated viruses. *Methods in Molecular Biology*, *737*, 247-278. doi: 10.1007/978-1-61779-095-9_11

# INVESTIGATOR STATEMENT

I have read the protocol, including all appendices, and I agree that it contains all of the necessary information for me and my staff to conduct this study as described. I will conduct this study as outlined herein, in accordance with Good Clinical Practice: Consolidated Guideline approved by the International Conference on Harmonization (ICH), National Institute of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) April 2016 and all applicable local and federal regulatory requirements and will make a reasonable effort to complete the study within the time designated.

I will provide all study personnel under my supervision copies of the protocol and any amendments, and access to all information provided by Spark or specified designees. I will discuss the material with them to ensure that they are fully informed about the study.

Principal Investigator (signature) Date (dd-MMM-yyyy)

Principal Investigator Name (printed) Site Number