GenSight Biologics

DISCLOSURE: REDACTED CLINICAL STUDY PROTOCOL VERSION 5.0

Title: Efficacy and Safety of Bilateral Intravitreal Injection of GS010: A Randomized, Double-Masked, Placebo-Controlled Trial in Subjects Affected with G11778A *ND4* Leber Hereditary Optic Neuropathy for Up to One Year

NCT Number: NCT03293524

Protocol Number: GS-LHON-CLIN-05

Clinical Study Protocol Version 5.0, Approval Date: 23-Dec-2019

Certain information within this protocol has been redacted (ie, specific content is masked irreversibly from view with a black bar) to protect either personally identifiable information or company confidential information.

This may include, but is not limited to, redaction of the following:

- Named persons or organizations associated with the study.
- Proprietary information, such as scales or coding systems which are considered confidential information under prior agreements with license holder.
- Other information as needed to protect confidentiality of GenSight Biologics or partners, personal information, or to otherwise protect the integrity of the clinical study.

The information contained in the following document is the property of GenSight Biologics and should not be shared or used for any purpose other than for which it was provided.

Clinical Research Protocol

Drug	GS010 (rAAV2/2-*ND4*)
Study Number	GS-LHON-CLIN-05
Protocol Title	Efficacy and Safety of Bilateral Intravitreal Injection of GS010: A Randomized, Double-Masked, Placebo-Controlled Trial in Subjects Affected with G11778A *ND4* Leber Hereditary Optic Neuropathy for Up to One Year
Study Name	REFLECT
Phase	III
Sponsor	GenSight Biologics 74 Rue de Faubourg Saint Antoine Paris 75012 France
Sponsor Contacts	[redacted] GenSight Biologics Phone: [redacted] Email: [redacted] [redacted] GenSight Biologics Phone: [redacted] Email: [redacted]
EudraCT Number	2017-002187-40
IND number	16538
NCT Number	NCT03293524
Original Protocol Date	15 June 2017
Version Number	5.0
Version Date	23 December 2019

Clinical Protocol Approval Form

Protocol Title	Efficacy and Safety of Bilateral Intravitreal Injection of GS010: A Randomized, Double-Masked, Placebo-Controlled Trial in Subjects Affected with G11778A *ND4* Leber Hereditary Optic Neuropathy for Up to One Year
Study Number	GS-LHON-CLIN-05
Original Protocol Date	15 June 2017
Protocol Version	5.0
Protocol Version Date	23 December 2019

This study protocol was subject to critical review and has been approved by the appropriate sponsor personnel. The information contained in this protocol is consistent with the current risk-benefit evaluation of the investigational product and the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki, and principles of Good Clinical Practice and according to applicable local requirements.

The Investigator will be supplied with details of any significant or new findings, including adverse events, relating to treatment with the investigational product.

Name and Title	Approval	Signature	Date
[redacted], GenSight Biologics	Yes No (circle one)		
[redacted]	Yes No (circle one)		

Study GS-LHON-CLIN-05

REFLECT

Efficacy Safety of Bilateral Intravitreal Injection of GS010: A Randomized, Double-Masked, Placebo-Controlled Trial in Subjects Affected with G11778A *ND4* Leber Hereditary Optic Neuropathy for Up to One Year

CONFIDENTIALITY AND INVESTIGATORS STATEMENT

The information contained in this protocol and all other information relevant to GenSight Biologics is the confidential and proprietary information of GenSight Biologics, and except as may be required by federal, state or local laws or regulation, may not be disclosed to others without prior written permission of GenSight Biologics.

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 the regulations stated in the guidelines for Good Clinical Practice and International Conference on Harmonization guidelines, 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 GenSight Biologics or specified designees. I will discuss the material with them to ensure that they are fully informed about GS010 and the study.

_____

Investigator Name

_____

Investigator Signature

_____ _____

Date Site Number

1. Study Synopsis

Study Title	Efficacy Safety of Bilateral Intravitreal Injection of GS010: A Randomized, Double-Masked, Placebo-Controlled Trial in Subjects Affected with G11778A *ND4* Leber Hereditary Optic Neuropathy for Up to One Year
Study Rationale	The Investigational Medicinal Product (IMP) GS010 is under development for the treatment of vision loss in Leber Hereditary Optic Neuropathy (LHON) due to the G11778A NADH dehydrogenase 4 gene (*ND4*) mitochondrial mutation. A dose-escalation, Phase I/II safety and tolerability study, GS-LHON-CLIN-01, determined the maximal well-tolerated dose to be [redacted] per eye. Two Phase III, randomized, sham-controlled trials of unilateral intravitreal GS010 administration are ongoing: GS-LHON-CLIN-03A RESCUE includes subjects with vision loss for up to six months and GS-LHON-CLIN-03B REVERSE includes subjects with vision loss for more than six months and up to 1 year. LHON is a bilateral disease. Often the eyes of a given subject are affected sequentially and potentially the elapsed time between onset of vision loss and treatment administration may impact the presence and magnitude of the treatment effect in a neurodegenerative disease such as LHON. As such, the next step in the clinical development plan for GS010 is to assess the efficacy and safety of bilateral administration of intravitreal GS010 and to determine if a difference exits in the treatment of the first and second affected or not yet affected eyes. Long-term safety will be assessed over a 5-year post treatment period.
Study Treatment	GS010 is a replication-defective, single stranded DNA recombinant Adeno-Associated Virus 2 (rAAV2) vector containing a codon modified complementary DNA (cDNA), encoding the human wild type mitochondrial NADH Dehydrogenase 4 protein (rAAV2/2-*ND4*), under the control of the cytomegalovirus immediate early promoter in an intron-containing expression cassette (beta globin intron, HBB2), flanked by the virus inverted terminal repeats. The GS010 (rAAV2/2-*ND4*) Drug Product is a sterile suspension of concentrated and purified virus vector formulated in Balanced Saline Solution (BSS) plus [redacted]. In this study subjects will receive an intravitreal (IVT) injection of GS010 in one or both eyes per the randomized treatment allocation. Each injection of GS010 will contain [redacted] per eye in a final volume of [redacted]. The placebo is a sterile, apyrogenic solution of Balanced Sterile Saline Solution, used for ocular surgery. In this study, eyes assigned to receive

	placebo intravitreal injection will receive a single injection of the placebo at a final volume of [redacted].
Study Population	The study population will include subjects with vision loss from LHON due to the G11778A point mutation in the mitochondrial *ND4* gene. Subjects with vision loss of up to 1 year in duration will be included.
Number of Subjects	Approximately 90 subjects (45 in each treatment group) will be included in the study. Depending on the number of subjects who terminate early, additional subjects may be enrolled through the end of the recruitment period.
Objectives	**Primary Objective:** To assess the efficacy of intravitreal GS010 compared to placebo intravitreal injection in second affected/not yet affected eyes, at 1.5 years post-treatment, analyzing the change from baseline of the best-corrected visual acuity (BCVA) reported with the Log of the Minimal Angle of Resolution (LogMAR), in *ND4* LHON subjects with vision loss up to one year. **Secondary Objectives:** The efficacy secondary objectives listed below will be analyzed for the second affected/not yet affected eyes as the main eye of interest (designated the primary eye). Exploratory efficacy analyses will also be conducted for the first affected eyes, but those analyses are secondary to any analysis for the primary eye, which is the second affected/not yet affected eye. 1. To assess the safety and tolerability of bilateral and unilateral intravitreal injection of GS010. 2. To compare the time course of the BCVA LogMAR response in second affected/not yet affected eyes treated with GS010 compared to placebo treatment. 3. To assess, at 1.5 years, 2, 3, 4 and 5 years post-treatment, the efficacy of intravitreal GS010 compared to placebo intravitreal injection in second affected/not yet affected eyes, by determining the difference in the rate of responder eyes. 4. To compare the time course of the response in second affected/not yet affected eyes treated with GS010 compared to placebo treatment and to estimate the magnitude of the treatment effect at 1.5 years, 2, 3, 4 and 5 years post-treatment, with parameters measured by: a. Spectral-Domain Optical Coherence Tomography (SD-OCT);

	b. Humphrey Visual Field (HVF) 30-2; c. Pelli-Robson Low Vision Contrast Sensitivity. 5. To verify whether a difference exists at 1.5 years, 2, 3, 4 and 5 years post-treatment, between first affected eyes and second affected/not yet affected eyes treated with GS010, using both an intra-subject and inter-subject analysis, with a. LogMAR BCVA b. Parameters measured by SD-OCT c. Parameters measured by HVF 30-2, and d. Parameters measured with Pelli Robson Low Vision Contrast Sensitivity. 6. To assess, at 1.5 years, 2, 3, 4 and 5 years post-treatment, the rate of responders in first affected eyes treated with GS010. 7. To assess humoral and cellular immune responses to AAV2 after unilateral and bilateral intravitreal administration. 8. To assess the impact of bilateral intravitreal GS010 administration on Quality of Life (QoL) scales at 1.5 years, 2, 3, 4 and 5 years post-treatment.
Study Design	GS-LHON-CLIN-05 is a Phase III, global, multi-center randomized, double-masked for the primary analysis, placebo-controlled, clinical study. As LHON is a neurodegenerative disease, the goal is to administer GS010 as soon as possible upon confirmation of the LHON diagnosis and the causative mutation. All subjects will receive a peri-treatment, systemic immune modulating regimen for the prevention or diminution of ocular inflammation related to IVT injection of GS010 (please see Section 13.9.1 for full details). At the Screening Visit (Visit1), the duration of vision loss of each eye of each subject will be determined and documented. The eye with the longest duration of vision loss will be the first affected eye and the eye with the shortest duration of vision loss will be the second affected eye. Eyes that have not yet experienced clinically manifested decline in visual acuity will have a value of zero days for vision loss duration and will be considered "not yet affected" eyes. As all subjects are required to have clinically manifested vision loss due to *ND4* LHON to any extent in at least one eye, each subject will thus have one eye designated as the "first affected" eye and one eye designated as the "second affected/not yet affected" eye. Subjects may report simultaneous onset of vision loss in both of their eyes, indicating true simultaneous onset of vision loss or inability of the subject to distinguish which is the first affected eye and which is the second affected eye. For these subjects, vision loss duration will be equal for both eyes.

	At the Inclusion Visit (Visit 2), the best-seeing and worst-seeing eye of each subject will be determined, based on the baseline vision testing performed at Visit 2. A pre-defined algorithm for determining the best- and worst-seeing eyes will be employed and is further detailed in section 13.10.1. An Interactive Response System (IRS) will assign a unique subject identification number at Screening Visit (Visit 1), which will be required for all communication between the Investigator (or designee) and the IRS. Subject numbers will be tracked via the IRS. The IRS will be used to enroll and randomize subjects based on a pre-defined central randomization scheme. An IRS User Guide will describe all steps for enrollment and randomization of subjects. Subjects meeting all eligibility criteria for selection and inclusion will be randomized to treatment arm 1 (TARM1) or treatment arm 2 (TARM2) in a 1:1 allocation (see Figure 1). Subjects in TARM1 will receive intravitreal GS010 in their first affected eye and their second affected/not yet affected eye. Subjects in TARM2 will receive intravitreal GS010 in their first affected eye and placebo intravitreal injection in their second affected/not yet affected eye. For subjects who report simultaneous onset of vision loss in both eyes, the right or left eye will be selected randomly to serve as the second affected/not yet affected eye. If the right eye is selected randomly as the second affected/not yet affected eye, the left eye will be designated as the first affected eye, and vice versa. Administration of an intraocular pressure (IOP) lowering agent of the investigator's choice will precede all intravitreal injections. Pre-intravitreal injection procedural preparation will include pupil dilation, topical antisepsis, and topical anesthesia. The IMPs (GS010 and placebo) will be administered with a standard intravitreal injection procedure. Each eye will undergo administration of the allocated treatment with a single intravitreal injection, performed as a separate procedure. Please refer to the Intravitreal GS010/Placebo Injection Guide for full details. Per the Investigator's discretion and according to local standard accepted medical practices, treatment administration to both eyes may be performed at a single Treatment Visit (Visit 3) (i.e. intravitreal injection of both eyes of a given subject is performed on the same day as two separate procedures) or may be performed on two consecutive days (i.e. one eye of the subject will be administered the allocated treatment one day and the next day the fellow eye of the subject will be administered the allocated treatment).

	Inclusion Visit (Visit 2) vision test results will serve as the baseline values for both eyes of a given subject when the treatments are administered on the same day and when they are administered on consecutive days. Study Day 0 is the day that treatment administration is completed to both eyes; in the setting of treatment administration on consecutive days, this will be the second treatment day. The timing of visits and visit windows for post-treatment follow-up visits will be determined from Study Day 0. The following rules must be adhered to: 1. Administration of the allocated treatment to both eyes of a given subject must be completed within two consecutive days after the day of the Inclusion Visit (Visit 2). 2. Follow-up (Visit 4) must be performed 1-day following the completion of treatment administration to both eyes. 3. The initial treatment administration must be performed the day following the Inclusion Visit (Visit 2). When both eyes are treated the same day, treatment must be performed the day after the Inclusion Visit (Visit 2). Please see Table 1 for treatment administration options. Masking will be accomplished with use of intravitreal injection of placebo. The pharmacy team will be the only study personnel/team not masked to treatment allocation (for the primary analysis). The treating and follow-up physicians and respective study teams will thus be masked to treatment allocation for second affected/not yet affected eye (i.e. the primary eye). Subject's will be double masked to treatment allocation. A Data Safety Monitoring Board (DSMB) will be convened for data and safety review at least every 6 months up to 2 years post-treatment. Each subject will be invited to participate in the Long-Term safety and efficacy follow-up (Post-Treatment Follow-up Period 2). Preferably, the informed consent of the Post-Treatment Follow-up Period 2 will be provided by Visit 13. **Study Visits/Periods:** The study is divided into 2 successive periods: 1) The first period covers enrollment, treatment and initial follow-up period of subjects up to 2 years Post-Treatment divided in 13 visits: a) Screening Visit (Visit 1) b) Inclusion Visit (Visit 2) c) Treatment Visit(s) (Visit(s) 3) d) Post-Treatment Follow-Up Period 1 (Visits 4 through 13, with 2 optional telephone visits)

	2) The second period, also called Post-Treatment Follow-Up Period 2 covers a long-term follow-up period, from 2 years to 5 years Post-Treatment divided in 3 additional visits (Visits 14, 15 and 16). • Screening Visit (Visit 1) Will occur from 28 to 7 days before the Treatment Visit(s) (Visit(s) 3). Informed consent signature will be obtained. Eligibility for selection will be assessed. Demographic and medical history will be obtained and general systemic evaluations (e.g. general physical examination, vital signs, 12-lead ECG) will be performed for screening and to obtain baseline values for comparison to post-treatment values. Laboratory assessments will be obtained. Vision loss duration of each eye will be determined, and vision testing will be performed per the study schedule. • Inclusion Visit (Visit 2) Will occur one day prior to the initial GS010/placebo administration. Eligibility will be confirmed based on the inclusion/exclusion criteria. Baseline visual evaluations and ophthalmological examinations will be performed for comparison to post-treatment visits. Baseline QoL scale assessment will be performed for comparison to post-treatment values. Best- and worst-seeing eyes will be determined prior to randomization. Duration of vision loss in the second affected eye will be documented if vision loss occurred after the Screening Visit (Visit 1). Upon confirmation of eligibility for inclusion, subjects will be randomized to the treatment arms based on a pre-defined central randomization scheme. • Treatment Visit(s) (Visit(s) 3) Subjects will receive the treatment as allocated based on the randomization. Per the Investigator's discretion and according to local standard accepted medical practices, treatment administration to both eyes may be performed at a single Treatment Visit 3 (i.e. intravitreal injection of both eyes of a given subject is performed the same day), or may be performed on two consecutive days (i.e. one eye of the subject will be administered the allocated treatment one day and the next day the fellow eye of the subject will be administered the allocated treatment). • Post-Treatment Follow-Up Period 1: Visits 4 through 13, with 2 optional telephone visits

	Subjects will attend ten follow-up visits conducted at Day 1, Week 2, Months 1, 2, 3, 6, and 9, and Years 1, 1.5, 2 post-treatment administration (in reference to Study Day 0). Each subject will be invited to participate in the Long-Term safety and efficacy follow-up (Post-Treatment Follow-up Period 2). If the subject was not provided with the informed consent form related to this follow-up at Visit 12 or at Visit 13, the Investigator will schedule a telephone visit, approximately 1 month before Visit 13 or approximately 2 weeks after Visit 13 respectively, to introduce the long-term follow-up period of the REFLECT study. • Post-Treatment Follow-Up Period 2: Visits 14 through 16 Subjects will attend 3 additional follow-up visits conducted at Year 3, 4 and 5 post-treatment administration (in reference to Study Day 0). A schedule of study events is presented in section 9.2. **Study Duration**: Initiation of the trial with the first subject's first visit occurred in March 2018. The total duration of study follow-up for each subject will be 5 years post-treatment. The Post-Treatment Follow-up Period 1 will end with the last subject's last visit 13 (Year 2) in Q3 2021. The study is expected to be completed with the last subject's last visit estimated to occur in Q3 2024 at the end of the Post-Treatment Follow-up Period 2. The estimated total duration of the study is six and a half years, including the time required to complete recruitment and all study visits for all subjects.
Primary Efficacy Endpoint	The primary efficacy endpoint will be the change from baseline (Visit 2) BCVA reported with LogMAR at 1.5 years post-treatment in second affected/not yet affected eyes of *ND4* LHON subjects with vision loss up to one year. The change from baseline (Visit 2) in second affected/not yet affected eyes receiving GS010 and placebo will be the primary response of interest. LogMAR BCVA will be used, to represent BCVA.
Secondary Efficacy Endpoints	All the following efficacy endpoints will be analyzed for the second affected/not yet affected eyes as the main eye of interest, but exploratory efficacy analyses will also be conducted for the first affected eyes. These analyses of the first affected eyes are considered secondary to any analysis of the primary eye which is the second affected/not yet affected eye. 1. Change from baseline in LogMAR BCVA at each timepoint of the follow-up period and at 2, 3, 4 and 5 years post-treatment.

	2. Response status at each timepoint of the follow-up period and at 2, 3, 4 and 5 years post-treatment. Definitions of responder eyes include: a. Eyes whose LogMAR BCVA improves (i.e. decreases) by ≥ 0.3 LogMAR (equivalent to a gain of ≥ 15 Early Treatment Diabetic Retinopathy Study (ETDRS) letters, compared to baseline. b. Eyes whose LogMAR BCVA does not increase (i.e. worsen) by ≥ 0.3 LogMAR (equivalent to eyes that lose ≤ 15 ETDRS letters) compared to baseline. c. Eyes whose LogMAR visual acuity is < 1.0 (i.e. better than LogMAR 1.0, equivalent to better than Snellen acuity of 20/200). 3. Change from baseline in parameters measured with SD-OCT (including the Ganglion Cell Layer (GCL) volume/thickness and the Temporal Quadrant of the Retina Nerve Fiber Layer (RNFL) thickness), HVF 30-2, and Pelli Robson Low Vision Contrast Sensitivity at each timepoint of the follow-up period and at 2, 3, 4 and 5 years post-treatment. 4. Change from baseline in visual Functioning Questionnaire-25 at each post-treatment visit. 5. Change from baseline in 36-Item Short Form Health Survey, version 2 Questionnaire at each post-treatment visit.
Safety Endpoints	1. Adverse events (AEs) and serious adverse events (SAEs), including those that are treatment-emergent and non-treatment-emergent, throughout the study period and at each study visit. Incidence and severity of systemic and local (ocular) AEs and SAEs will be determined at each clinical site and for the entire study cohort. 2. Results of physical examinations, vital signs, electrocardiograms (ECGs), and laboratory results for hematology and serum chemistry tests during the study. 3. Results of immune response evaluations: a. Time course of the humoral immune response measured with Neutralizing Antibodies (NAb) against AAV2 b. Time course of the cellular immune response against AAV2 4. Results of bio-dissemination testing up to 4 weeks post-treatment.

2. Contents

3. List of Tables

4. List of Figures

5. List of Abbreviations

AAV / AAV2	Adeno-associated virus / Adeno-associated virus serotype 2
AE	Adverse Event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AR(1)	First-order Autoregressive
ARH(1)	Heterogeneous First-order Autoregressive
AST	Aspartate aminotransferase
ATC	Anatomical therapeutic chemical
ATP	Adenosine triphosphate
AU	Arbitrary units
AUC	Area Under the Curve
BCVA	Best Corrected Visual Acuity
BP	Blood Pressure
BSS	Balance Sterile Saline Solution
BUN	Blood urea nitrogen
cDNA	complementary DNA
CF	Count fingers
CFR	Code of Federal Regulations
CI	Complex I
CI	Confidence interval
CLIN-01	GS-LHON-CLIN-01
CLIN-03A	GS-LHON-CLIN-03A RESCUE
CLIN-03B	GS-LHON-CLIN-03B REVERSE
CMH	Cochran-Mantel-Haenszel
CMO	Contract Manufacturing Organization
CRA	Clinical Research Associate
CRO	Contract Research Organization

CS	Compound Symmetry
CSH	Heterogeneous Compound Symmetry
CTCAE	Common Terminology Criteria for Adverse Events
eCRF	Electronic Case Report Form
ELISA	Enzyme-linked immunosorbent assay
ELIspot	Enzyme-linked immunospot assay
EMA	European Medicines Agency
ETDRS	Early Treatment Diabetic Retinopathy Study
EU	European Union
FA	Fluorescein Angiography
FDA	Food and Drug Administration (United States)
GCL	Ganglion cell layer
GCP	Good Clinical Practice
γGT	Gamma-glutamyl transpeptidase
GMO	Genetically Modified Organism
HIV	Human immunodeficiency virus
HM	Hand motion
HVF	Humphrey visual field
IATA	International Air Transport Association
IC_{50}	Half-maximal inhibitory concentration
ICF	Informed Consent Form
ICH	International Council on Harmonization
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
IFNγ	Interferon gamma
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
IOP	Intraocular pressure
IRB	Institutional Review Board

IRS	Interactive Response System
ITT	Intention-To-Treat
IVT	Intravitreal
LHON	Leber (or Leber's) Hereditary Optic Neuropathy
LP	Light perception
LogMAR	Logarithm of the minimal angle of resolution
LS	Least square
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligrams
mITT	Modified Intention-To-Treat
mtDNA	Mitochondrial DNA
Nab	Neutralizing antibodies
ND4	NADH dehydrogenase 4 gene
NHP	Non-human primates
NLP	No light perception
P.O.	Per os (i.e. orally)
PP	Per Protocol
Q1	First quartile
Q3	Third quartile
QoL	Quality of Life
qPCR	Quantitative polymerase chain reaction
rAAV2	Recombinant adeno-associated virus 2
rAAV2/2-*ND4*	recombinant adeno-associated virus 2 vector, containing a modified cDNA encoding the human wild-type mitochondrial NADH dehydrogenase 4 protein
RGC	Retinal Ganglion Cell
RNFL	Retinal nerve fiber layer
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation

SD-OCT	Spectral-domain optical coherence tomography
SF-36v2	36-Item Short Form Health Survey Version 2
SLE	Slit lamp examination
SOP	Standard Operating Procedure
TARM1	Treatment arm 1
TARM2	Treatment arm 2
TBD	To be determined
TEAE	Treatment Emergent Adverse Event
TOEP	Toeplitz
TOEPH	Heterogeneous Toeplitz
US	United States
VFQ-25	Visual Functioning Questionnaire-25
vg	Viral genomes
WHO	World Health Organization

6. Introduction and Rationale

6.1 Leber Hereditary Optic Neuropathy and GS010

Leber Hereditary Optic Neuropathy (LHON) is a rare, maternally inherited mitochondrial genetic disease, typically presenting as a non-syndromic optic neuropathy. Retinal ganglion cells (RGC), whose axons form the optic nerve, are the major cellular target affected by the resultant mitochondrial dysfunction. LHON was the first inherited human disease associated with point mutations in the mitochondrial DNA (mtDNA) (Wallace 1988) and it is considered the most common inherited genetic mitochondrial disorder (Man 2002). LHON is a rare disease and in Northern Europe an estimated prevalence of between 1 in 30,000 to 1 in 50,000 has been reported (Yu-Wai-Man 2011, Puomila 2007, Spruijt 2007). In the northeast of the United Kingdom there is a reported prevalence of vision loss of 3.22 per 100,000 and it is estimated that 2% of Australians less than 65 years of age who are registered as legally blind have vision loss from LHON (Newman 2005a).

Three primary point mutations in the mtDNA are causative of LHON in approximately 90% of those affected. These point mutations, G3460A, G11778A and T14484C, occur respectively in the *ND1*, *ND4* and *ND6* genes of the mtDNA and all encode protein subunits of Complex I (CI) of the mitochondrial respiratory chain. The resultant dysfunction of CI and the respiratory chain lead to abnormal mitochondrial metabolic function, decreased adenosine triphosphate (ATP) production and increased reactive oxygen species. This leads to cell death of the RGC and resulting atrophy of the optic nerve (Yu-Wai-Man 2011). In Northern European and Australian populations, the G11778A *ND4* mutation is found in approximately 70% of subjects presenting with LHON related vision loss (Man 2002, Wallace 1988, Newman 2005a). Asian populations, for example the Japanese, have an even greater frequency of the *ND4* mutation, with up to 90% of Japanese subjects reported to have the G11778A *ND4* mutation (Nakamura 1993, Mashima 1998).

Men are affected more commonly than women, with a male predominance of greater than 80% reported in most pedigrees (Newman 2004). Classically, males are affected between ages 15 and 35 years, but typical LHON has been reported at almost any age, from 2 to 87 years old, in both men and women (Newman 2005a, Barboni 2006, Yu-Wai-Man 2009).

LHON manifests as acute to sub-acute, sequential, bilateral, painless vision loss. It is a bilateral disease and clinical manifestation is essentially predictive of bilateral involvement. Greater than 97% of subjects are reported to have bilateral involvement at 12 months and occurrences of unilateral involvement in retrospective reports of cohorts with LHON are very rare (Newman 1991, Riordan-Eva 1995). Lam et al. (Lam 2014) reported a G11778A *ND4* cohort in which 53% reported bilateral involvement in ≤ 2 months and 80% reported bilateral involvement in ≤ 6 months.

The clinical hallmark of LHON is central vision loss (Sadun 2011). This is accompanied by dysfunction of color and contrast vision and central and cecocentral visual field defects. Newman et al. (Newman 1991) reported a final visual acuity of 20/200 or worse in 98% of eyes. Lam et al. (Lam 2010, Lam 2014) performed a prospective natural history study of LHON subjects with the G11778A *ND4* mutation. The primary outcome was the finding of no significant difference in

ETDRS visual acuity at 12, 24 or 36 months of follow-up compared to baseline at study entry. This finding was for the entire cohort as well as for the subgroups of subjects who entered the study within 12 months of disease onset or after 12 months of disease onset. 14.7% of total study eyes, in 18% of study subjects, had improvement by ≥15 ETDRS letters over the study period. Eighty-six percent of subjects had eyes with stable acuities during study follow-up. Subjects entering the study after 36 months from disease onset showed no change or improvement of visual acuity over follow-up. The authors concluded that for G11778A *ND4* mutation spontaneous natural recovery is partial and occurs on a very limited basis in a small percentage of subjects and thus gene therapy interventions can be measured for efficacy with a reasonable sample size.

Currently there is no approved therapeutic agent for the treatment of vision loss from LHON in the United States. The European Medicines Agency (EMA) recommended a centralized marketing approval under exceptional circumstances for Raxone® (Idebenone) (Raxone® European Product Assessment Report 2015). Klopstock et al. (Klopstock 2011) studied idebenone for the treatment of vision loss from LHON caused by the three most common primary mutations (*ND1*, *ND4*, *ND6*); the study failed to reach its primary endpoint. Treatment options for vision loss from LHON remain limited and no therapies aimed at reversal of the molecular defect are available.

The Investigational Medicinal Product (IMP) GS010, is a replication-defective single stranded DNA recombinant Adeno-Associated Virus 2 (rAAV2) vector containing a codon modified complementary DNA (cDNA), encoding the human wild type mitochondrial NADH Dehydrogenase 4 protein (rAAV2/2-*ND4)*, under the control of the cytomegalovirus immediate early promoter in an intron-containing expression cassette (beta globin intron, *HBB2*), flanked by the virus inverted terminal repeats from AAV2/2. GS010 is under development for the treatment of vision loss in LHON when due to the G11778A *ND4* mitochondrial mutation.

6.2 Study Rationale

The following sections highlight the rationale for the major components of this protocol.

6.2.1 Clinical Development Program for GS010

GS010 is being developed for the treatment of vision loss in LHON due to the G11778A *ND4* mitochondrial mutation. The clinical development of GS010 has included:

- Safety and tolerability study with dose-exploration

GS-LHON-CLIN-01: A Phase I/II, open label, dose-escalation safety and tolerability study. The maximal well-tolerated dose of [redacted] per eye was selected for further study.

- Unilateral administration efficacy study in subjects with vision loss up to six months

GS-LHON-CLIN-03A RESCUE: A Phase III randomized, sham-controlled, GS010 (dose [redacted]) administration study in subjects with vision loss for up to six months.

- Unilateral administration efficacy study in subjects with vision loss for more than six months and up to 1 year

GS-LHON-CLIN-03B REVERSE: A Phase III randomized, sham-controlled, GS010 (dose [redacted]) administration study in subjects with vision loss for more than six months and up to 1 year.

LHON is a bilateral disease. Often the eyes of a given subject are affected sequentially and potentially the elapsed time between onset of vision loss and treatment administration may impact the presence and magnitude of the treatment effect in a neurodegenerative disease such as LHON. As such, the next step in the clinical development plan for GS010 is to assess the safety and efficacy of bilateral administration of intravitreal GS010 and to determine if a difference exits in the treatment of the first- and second-affected or not-yet-affected eyes.

Recent GS010 study results have shown that the benefit from GS010 is more pronounced after 1-year post injection. Preliminary data from the REVERSE study demonstrated that there is an improvement from baseline in BCVA at Week 72 of +15 ETDRS letters (- 0.295 [Standard Error ±0.063] LogMAR; $p < 0.0001$) and of +12 ETDRS letters (-0.246 [Standard Error ±0.063] LogMAR; $p = 0.0003$) in GS010 treated eyes and sham treated eyes, respectively (data on file). This improvement in BCVA was less marked at Week 48 with +11 letters and +10 letters in GS010 treated eyes and sham eyes, respectively (data on file). This sustained and increased improvement in BCVA over time supports a read out of the primary endpoint in the REFLECT study at 1.5 year-post treatment.

6.2.2 Selection of the Primary Eye for Comparison and Randomization Scheme

LHON is a neurodegenerative disease with apoptotic neuronal cell death of the RGC. Structural imaging with Optical Coherence Tomography has revealed a progressive loss of the Ganglion Cell Layer (GCL) and Retinal Nerve Fiber Layer (RNFL), commencing in the pre-symptomatic stage through the acute and sub-acute stages of vision loss (Barboni 2010, Balducci 2016, Hedges 2016, Moster 2016).

The bilateral nature and common manifestation of sequential onset of vision loss have also enabled a unique opportunity to study investigational therapeutics in LHON. The paradigm of therapeutic intervention prior to clinically manifested vision loss in the second eye to be affected has been recommended as powerful method of proving therapeutic effectiveness in LHON (Newman 2005). Lam and colleagues (Lam 2010, Lam 2014) are developing an alternative vector for gene delivery in LHON *ND4* and recommend that efficacy should be proven in recently affected subjects with Snellen visual acuity of better than 20/40 in one eye and worse than 20/40 in the fellow eye, with active treatment administered to the better seeing eye. Klopstock and colleagues (Klopstock 2011) performed a randomized controlled trial of idebenone (Raxone®) and although the study failed to reach its primary endpoint, a trend of response was noted in subjects with discordant visual acuities, in essence, those subjects with relatively preserved visual acuity in one eye and loss of visual acuity in the other eye.

In this study protocol, the eye selected for primary analysis is the second affected/not yet affected eye. The study design will also allow assessment of the presence of a difference in treatment effect in first affected and secondly affected/not yet affected eyes, with an intra-subject comparison.

Subjects will be randomized to one of two treatment arms. In both treatment arms, the first affected eyes will receive intravitreal GS010. The two arms differ in that secondly affected/not yet affected eyes will receive intravitreal GS010 in one treatment arm and in the other treatment arm, secondly affected/not yet affected eyes will receive a placebo intravitreal injection. When subjects report simultaneous onset of vision loss in both of their eyes, indicating that the subject was unable to distinguish which is the first affected eye and which is the second affected/not yet affected eye, the second affected/not yet affected eye will be chosen randomly to minimize bias.

6.2.3 GS010 Dose for Administration

The maximal, well-tolerated dose, as determined from the Phase I/II GS-LHON-CLIN-01 study, is [redacted] This dose is being further studied in the unilateral administration Phase III studies RESCUE and REVERSE. It is intended to administer [redacted] in a final volume of [redacted] in this study.

6.2.4 Placebo Intravitreal Injection

This study includes a treatment arm (Treatment ARM2) in which each subject will receive intravitreal GS010 in their first affected eye and placebo intravitreal injection in their second affected/not yet affected eye. To appropriately mask subjects and the investigation team and minimize bias, a placebo intravitreal injection will be utilized. Please see Section 13.7.2 for a full description of the placebo injection.

6.2.5 Administration of an IOP lowering Agent Prior to Intravitreal Injection

IVT injection of therapeutic agents is associated with elevation of the IOP (Hollands 2007, Bakri 2009, Wu 2009, Frenkel 2010, Abedi 2013, El Chehab 2013, Kim 2013, Katayama 2014, Murray 2014, Dedania 2015). Data from ongoing clinical trials of intravitreal GS010 administration indicates that elevation of IOP is an expected potential side effect after intravitreal GS010 injection (please see refer to the Investigator's Brochure for additional details).

Use of pre-IVT injection IOP lowering agents has been shown by some groups to be effective in reducing the IOP elevation (Kim 2013). Others have recommended pre-treatment with IOP lowering agents in subjects whose optic nerve is at risk for further damage from transient or sustained IOP elevation, such as glaucoma subjects (Abedi 2013). By extension, the optic nerve of LHON subjects may be at higher risk for damage from transient or sustained IOP elevation, due to its already compromised status. Therefore, subjects will undergo administration of an investigator selected IOP lowering agent pre-IVT injection of GS010 and pre-IVT injection of placebo.

6.2.6 Administration of Immune Modulating Regimen

Immune responses to Adeno-associated virus (AAV) capsid proteins or the transgene product are reported to occur (Bennett 2003, Kumar-Singh 2008). Data in animal studies reveal a time- and

dose-dependent increase in neutralizing antibodies against AAV (MacLachlan 2011; Koilkonda 2014; Kotterman 2015). Several reports document ocular inflammation in pre-clinical studies of intravitreal AAV injection (MacLachlan 2011, Ye 2015, Ramachandran 2016).

GenSight-sponsored pre-clinical NHP studies of intravitreal GS010 also revealed the occurrence of ocular inflammation (Bouquet et al. in preparation). Thus, based on the available medical and scientific literature and the preclinical experience with intravitreal GS010, ocular inflammation is an expected adverse side effect of intravitreal GS010 injection. The occurrence of ocular inflammation after intravitreal injection of GS010 as a common ocular adverse side effect has also been noted in human clinical studies of GS010 to date. Please see the Investigator's Brochure for full details. To date, no trial of GS010 has been performed utilizing a peri-treatment immune modulating regimen.

Two other clinical programs are currently studying intravitreal AAV in subjects with vision loss due *ND4* LHON. The first group (Wan 2016, Yang 2016) utilized oral corticosteroid immune modulation to prevent immune response and ocular inflammation and reported no ocular inflammation or any adverse events. The second group (Feuer 2016, Guy 2016 ARVO) reported preliminary results of a trial utilizing intravitreal self-complementary AAV, in which no immune modulation was utilized and noted the presence of low-grade ocular inflammation. This group had previously reported mild vitritis in a monkey (Koilkonda 2014).

Several ocular gene therapy studies have employed peri-treatment immune modulation to prevent ocular inflammation when administering intravitreal or sub-retinal AAV-based therapies (Bainbridge 2008, Hauswirth 2008, Maguire 2008, Cideciyan 2009, Maguire 2009, Bennett 2012, Jacobson 2012, MacLaren 2014, Bainbridge 2015, Rakoczy 2015).

Based on the medical and scientific literature noted above, preclinical NHP data for intravitreal GS010 injection, current experience in human clinical trials of GS010, and expert clinician recommendations, an immune-modulating regimen will be administered into subjects participating in this clinical trial. Please see sections 9 and 13.9.1 for additional details.

6.2.7 Long-term Risk Assessment Management

According to the EMA Committee for Medicinal Products for Human Use Guideline, entitled "Follow-Up of Patients Administered with Gene Therapy Medicinal Products," and the United States Food and Drug Administration (US FDA) Center for Biologics Evaluation and Research Guidance for Industry (2006) entitled, "Gene Therapy Clinical Trials: Observing Subjects for Delayed Adverse Events," viral vectors without integration, latency, or reactivation potential like AAV vectors present a low risk for gene therapy-related delayed adverse reactions. Although the FDA indicated that long-term follow-up is not considered necessary for this clinical development program, the EMA recommends a yearly monitoring plan up to 5 years post-treatment.

The regulatory intention of further observation is to determine the sustainability of efficacy, or whether declining efficacy after administration of gene therapy IMP occurs or whether more time is needed for tissue to be fully functional. For safety, the intention is to determine delayed-onset of adverse events (AEs), and to determine any additional unexplored long-term risks. The EMA refers to the Guideline (2008) entitled "Safety and Efficacy Follow-up: Risk Management of Advanced

Therapy Medicinal Products" for possible delayed risks.

For these reasons, the follow-up period of this study is being extended from 2 years to 5 years.

6.3 Preclinical Studies

Preclinical non-human primate studies supporting the clinical development of GS010 include:

- A single intravitreal administration of GS010 into one or both eyes of NHP; and
- A delayed, bilateral administration of GS010 into both eyes of NHP.

Please refer to the current version of the Investigator's Brochure for a summary of the results of these studies and the Investigational Medicinal Product Dossier (IMPD), for full details.

6.4 Clinical Safety of GS010

6.4.1 Adverse Event Profile

The safety and tolerability of GS010 has been developed from data from all human clinical trials studying intravitreal GS010 administration. Please refer to the current version of the Investigator's Brochure for complete details.

6.4.2 Bio-Distribution and Shedding

Data obtained from the GS-LHON-CLIN-01 study, confirm the very limited risk of bio-dissemination in humans. For additional details please see the current version of the Investigator's Brochure.

6.4.3 Immunogenicity

Immune responses against AAV2 are expected. Immune responses against AAV2 are being studied in the GS-LHON-CLIN-01, RESCUE and REVERSE studies. Please refer to the current version of the Investigator's Brochure for full details.

6.4.4 Potential Drug-Drug Interactions

GS010 is not distributed to or metabolized by the liver. Therefore drug-drug interactions are unlikely.

7. Study Objective(s)

7.1 Primary Objective

To assess the efficacy of intravitreal GS010 compared to placebo intravitreal injection in second affected/not yet affected eyes, at 1.5 years post-treatment, analyzing the change from baseline of the BCVA reported with LogMAR, in *ND4* LHON subjects with vision loss up to one year.

7.2 Secondary Objective(s)

The efficacy objectives listed below will be analyzed for the second affected/not yet affected eyes as the main eye of interest (designated the primary eye). Exploratory efficacy analyses will also be conducted for the first affected eyes, but those analyses are secondary to any analysis for the primary eye which is the second affected/not yet affected eye.

1. To assess the safety and tolerability of bilateral and unilateral intravitreal injection of GS010.
2. To compare the time course of the BCVA LogMAR response in second affected/not yet affected eyes treated with GS010 compared to placebo treatment.
3. To assess, at 1.5 years, 2, 3, 4 and 5 years post-treatment, the efficacy of intravitreal GS010 compared to placebo intravitreal injection in second affected/not yet affected eyes, by determining the difference in the rate of responder eyes.
4. To compare the time course of the response in second affected/not yet affected eyes treated with GS010 compared to placebo treatment and to estimate the magnitude of the treatment effect at 1.5 years, 2, 3, 4 and 5 years post-treatment, with parameters measured by:
    a. SD-OCT;
    b. HVF 30-2;
    c. Pelli-Robson Low Vision Contrast Sensitivity.
5. To verify whether a difference exists at 1.5 years 2, 3, 4 and 5 years post-treatment, between first affected eyes and second affected/not yet affected eyes treated with GS010, using both an intra-subject and inter-subject analysis, with
    a. LogMAR BCVA
    b. Parameters measured by SD-OCT
    c. Parameters measured by HVF 30-2, and
    d. Parameters measured with Pelli Robson Low Vision Contrast Sensitivity.
6. To assess, at 1.5 years, 2, 3, 4 and 5 years post-treatment, the rate of responders in first affected eyes treated with GS010.
7. To assess humoral and cellular immune responses to AAV2 after unilateral and bilateral intravitreal administration.
8. To assess the impact of bilateral intravitreal GS010 administration on QoL scales at 1.5, 2, 3, 4 and 5 years post-treatment.

8. Study Endpoint(s)

8.1 Primary Efficacy Endpoint

The primary efficacy endpoint will be the change from baseline (Visit 2) BCVA reported with LogMAR at 1.5 years post-treatment in second affected/not yet affected eyes of *ND4* LHON subjects with vision loss up to one year. The change from baseline (Visit 2) in second affected/not yet affected eyes receiving GS010 and placebo will be the primary response of interest. LogMAR BCVA will be used to represent BCVA.

8.2 Secondary Efficacy Endpoint(s)

All the following efficacy endpoints will be analyzed for the second affected/not yet affected eyes as the main eye of interest, but exploratory efficacy analyses will also be conducted for the first affected eyes. These analyses of the first affected eyes are considered secondary to any analysis for the primary eye which is the second affected/not yet affected eye.

1. Change from baseline in LogMAR BCVA at each timepoint of the follow-up period and at 2, 3, 4 and 5 years post-treatment.
2. Response status at each timepoint of the follow-up period and at 2, 3, 4 and 5 years post-treatment. Definitions of responder eyes include:
   a. Eyes whose LogMAR BCVA improves (i.e. decreases) by ≥ 0.3 LogMAR (equivalent to a gain of ≥ 15 ETDRS letters) compared to baseline.
   b. Eyes whose LogMAR BCVA does not increase (i.e. worsen) by ≥ 0.3 LogMAR (equivalent to eyes that lose ≤ 15 ETDRS letters) compared to baseline.
   c. Eyes whose LogMAR visual acuity is < 1.0 (i.e. better than LogMAR 1.0, equivalent to better than Snellen acuity of 20/200).
3. Change from baseline in parameters measured with SD-OCT (including the GCL volume and the Temporal Quadrant RNFL thickness), HVF 30-2, and Pelli Robson Low Vision Contrast Sensitivity at each timepoint of the follow-up period and at 2, 3, 4 and 5 years post-treatment.
4. Change from baseline in Visual Functioning Questionnaire-25 at each post-treatment visit.
5. Change from baseline in 36-Item Short Form Health Survey, version 2 Questionnaire at each post-treatment visit.

8.3 Safety Endpoint(s)

1. Adverse Events (AEs) and Serious Adverse Events (SAEs), including those that are treatment-emergent and non-treatment-emergent, throughout the study period and at each study visit. Incidence and severity of systemic and local (ocular) AEs and SAEs will be determined at each clinical site and for the entire study cohort.
2. Results of physical examinations, vital signs, electrocardiograms (ECGs), and laboratory results for hematology and serum chemistry tests during the study.
3. Results of immune response evaluations:
   a. Time course of the humoral immune response measured with NAb against AAV2
   b. Time course of the cellular immune response against AAV2
4. Results of bio-dissemination testing up to 4 weeks post-treatment.

9. Study Plan

9.1 Study Design

GS-LHON-CLIN-05 is a Phase III, global, multicenter, randomized, double-masked for the primary analysis, placebo-controlled, clinical study. As LHON is a neurodegenerative disease, the goal is to administer GS010 as soon as possible upon confirmation of the LHON diagnosis and the causative mutation.

All subjects will receive a peri-treatment, systemic immune modulating regimen for the prevention or diminution of ocular inflammation related to IVT injection of GS010 (please see Section 13.9.1 for full details).

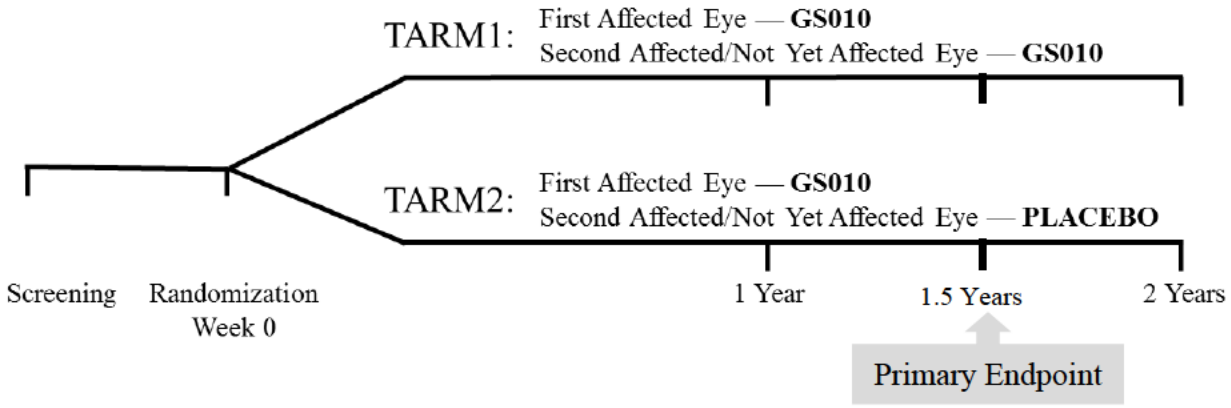
At the Screening Visit (Visit 1), the duration of vision loss of each eye of each subject will be determined and documented. The eye with the longest duration of vision loss will be the first affected eye and the eye with the shortest duration of vision loss will be the second affected eye. Eyes that have not yet experienced clinically manifested decline in visual acuity will have a value of zero days for vision loss duration and will be considered "not yet affected" eyes. As all subjects are required to have clinically manifested vision loss due to *ND4* LHON to any extent in at least one eye, each subject will thus have one eye designated as the "first affected" eye and one eye designated as the "second affected/not yet affected" eye. Subjects may report simultaneous onset of vision loss in both of their eyes, indicating true simultaneous onset of vision loss or inability of the subject to distinguish which is the first affected eye and which is the second affected eye. For these subjects, vision loss duration will be equal for both eyes.

At the Inclusion Visit (Visit 2), new onset vision loss occurring since the Screening Visit (Visit 1) in an eye previously unaffected by clinically manifested visual acuity loss will be documented and vision loss duration will be recorded. The best-seeing and worst-seeing eye of each subject will be determined, based on the baseline vision testing performed at Visit 2. A pre-defined algorithm, detailed in Section 13.10.1, to determine the best- and worst-seeing eyes will be employed.

An Interactive Response System (IRS) will assign a unique subject identification number at Screening Visit (Visit 1), which will be required for all communication between the Investigator (or designee) and the IRS. Subject numbers will be tracked via the IRS. The IRS will be used to enroll and randomize subjects based on a pre-defined central randomization scheme. An IRS User Guide will describe all steps for enrollment and randomization of subjects.

Subjects meeting all eligibility criteria for selection and inclusion will be randomized to treatment arm 1 (TARM1) or treatment arm 2 (TARM2) in a 1:1 allocation (see Figure 1). Subjects in TARM1 will receive intravitreal GS010 in their first affected eye and their second affected/not yet affected eye. Subjects in TARM2 will receive intravitreal GS010 in their first affected eye and placebo intravitreal injection in their second affected/not yet affected eye. For subjects who report simultaneous onset of vision loss in both eyes, the right or left eye will be selected randomly to serve as the second affected/not yet affected eye. If the right eye is selected randomly as the second affected/not yet affected eye, the left eye will be designated as the first affected eye, and vice versa.

Figure 1: Post-Treatment Follow-up Period 1: up to 2 years post-treatment



Administration of an IOP lowering agent of the investigator's choice will precede all intravitreal injections. Pre-intravitreal injection procedural preparation will include pupil dilation, topical antisepsis, and topical anesthesia. The IMPs (GS010 and placebo) will be administered with a standard intravitreal injection procedure. Each eye will undergo administration of the allocated treatment with a single intravitreal injection, performed as a separate procedure. Please refer to the Intravitreal GS010/Placebo Injection Guide for full details.

Per the Investigator's discretion and according to local standard accepted medical practices, treatment administration to both eyes may be performed at a single Treatment Visit (Visit 3) (i.e. intravitreal injection of both eyes of a given subject is performed the same day as two separate procedures) or may be performed on two consecutive days (i.e. one eye of the subject will be administered the allocated treatment one day and the next day the fellow eye of the subject will be administered the allocated treatment).

Inclusion Visit (Visit 2) vision test results will serve as the baseline values for both eyes of a given subject when the treatments are administered on the same day and when they are administered on consecutive days. Study Day 0 is the day that treatment administration is completed to both eyes; in the setting of treatment administration on consecutive days, this will be the second treatment day. The timing of visits and visit windows for post-treatment follow-up visits will be determined from Study Day 0. The following rules must be adhered to:

1. Administration of the allocated treatment to both eyes of a given subject must be completed within two consecutive days after the day of the Inclusion Visit (Visit 2).
2. Follow-up (Visit 4) must be performed 1-day following the completion of treatment administration to both eyes.
3. The initial treatment administration must be performed the day following the Inclusion Visit (Visit 2). When both eyes are treated the same day, treatment must be performed the day after the Inclusion Visit (Visit 2).

Table 1 describes the two options for treatment administration timing.

Table 1: Treatment Administration Options

OPTION 1 (Eyes are treated on 2 consecutive days)		
Day -2	**Day -1**	**Day 0**
Inclusion	First IVT injection in one eye	Second IVT injection in fellow eye

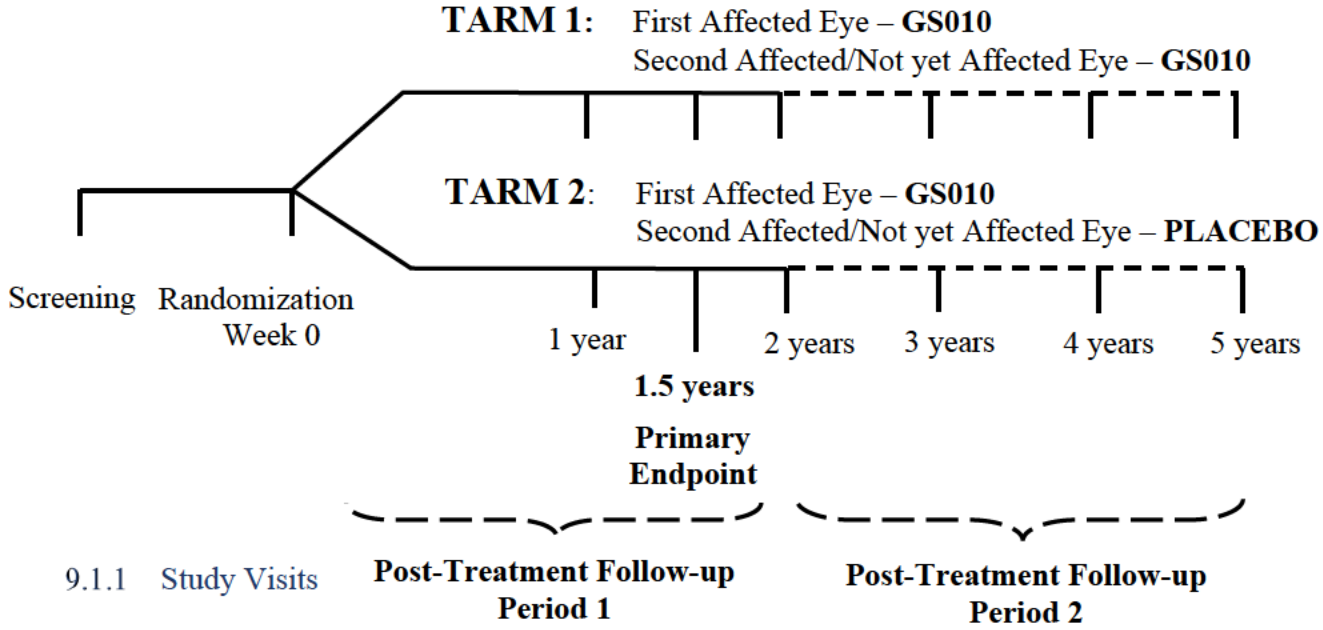
OPTION 2 (Eyes are treated on the same day)	
Day -1	**Day 0**
Inclusion	Two IVT injections (one in each eye)

Masking will be accomplished with use of intravitreal injection of placebo. The pharmacy team will be the only study personnel/team not masked to treatment allocation (for the primary analysis). The treating and follow-up physicians and respective study teams will thus be masked to treatment allocation for second affected/not yet affected eye (i.e. the primary eye). Subjects will be double masked to treatment allocation.

A Data Safety Monitoring Board (DSMB) will be convened for data and safety review at least every 6 months up to 2 years post-treatment.

Each subject will be invited to participate in the Long-Term safety and efficacy follow-up (Post-Treatment Follow-up Period 2). Preferably, the informed consent of the Post-Treatment Follow-up Period 2 will be provided by V13.

Figure 2: Post-Treatment Follow-up Periods 1 and 2: up to 5 years post-treatment



9.1.1 Study Visits

The study is divided into 2 successive periods:

1) The first period covers enrollment, treatment and initial follow-up period of subjects up to 2 years Post-Treatment divided in 13 visits:
   a) Screening Visit (Visit 1)
   b) Inclusion Visit (Visit 2)
   c) Treatment Visit(s) (Visit(s) 3)
   d) Post-Treatment Follow-Up Period 1 (Visits 4 through 13, with 2 optional telephone visits)

2) The second period, also called Post-Treatment Follow-Up Period 2 covers a long term follow-up period, from 2 years to 5 years Post-Treatment divided in 3 additional visits (Visits 14, 15 and 16).

- Screening Visit (Visit 1)

Will occur from 28 to 7 days before the Treatment Visit(s) (Visit(s) 3). Informed consent signature will be obtained. Eligibility for selection will be assessed. Demographic and medical history will be obtained and general systemic evaluations (e.g. general physical examination, vital signs, 12-lead ECG) will be performed for screening and to obtain baseline values for comparison to post-treatment values. Laboratory assessments will be obtained. Vision loss duration of each eye will be determined and vision testing will be performed per the study schedule.

- Inclusion Visit (Visit 2)

Will occur one day prior to the initial GS010/placebo administration. Eligibility will be confirmed based on the inclusion/exclusion criteria. Baseline visual evaluations and ophthalmological examinations will be performed for comparison to post-treatment visits. Baseline QoL scale assessment will be performed for comparison to post-treatment values. Best- and worst-seeing eyes will be determined prior to randomization. Duration of vision loss in the second affected eye will be documented if vision loss occurred after the Screening Visit (Visit 1).

Upon confirmation of eligibility for inclusion, subjects will be randomized to the treatment arms based on a pre-defined central randomization scheme.

- Treatment Visit(s) (Visit(s) 3)

Subjects will receive the treatment as allocated based on the randomization. Per the Investigator's discretion and according to local standard accepted medical practices, treatment administration to both eyes may be performed at a single Treatment Visit 3 (i.e. intravitreal injection of both eyes of a given subject is performed the same day), or may be performed on two consecutive days (i.e. one eye of the subject will be administered the allocated treatment one day and the next day the fellow eye of the subject will be administered the allocated treatment).

- Post-Treatment Follow-Up Period 1: Visits 4 through 13, with 2 optional telephone visits

Subjects will attend ten follow-up visits conducted at Day 1, Week 2, Months 1, 2, 3, 6, and 9, and Years 1, 1.5, and 2 post-treatment administration (in reference to Study Day 0).

Each subject will be invited to participate in the Long-Term safety and efficacy follow-up (Post-Treatment Follow-up Period 2). If the subject was not provided with the informed consent form related to this follow-up at Visit 12 or at Visit 13, the Investigator will schedule a telephone visit, approximately 1 month before Visit 13 or approximately 2 weeks after Visit 13 respectively, to introduce the long-term follow-up period of the REFLECT study.

- Post-Treatment Follow-Up Period 2: Visits 14 through 16

Subjects will attend 3 additional follow-up visits conducted at Year 3, 4 and 5 post-treatment administration (in reference to Study Day 0).

A schedule of study events is presented in section 9.2.

9.1.2 Study duration

Initiation of the trial with the first subject's first visit occurred in March 2018. The total duration of study follow-up for each subject will be 5 years post-treatment. The Post-Treatment Follow-up Period 1 will end with the last subject's last visit 13 (Year 2) in Q3 2021. The study is expected to be completed with the last subject's last visit estimated to occur in Q3 2024. The estimated total duration of the study is six and a half years, including the time required to complete recruitment, all study visits for all subjects.

At the completion of this study, all participating subjects will be invited/encouraged to participate in a long-term safety follow-up study as detailed in a separate protocol. The long-term safety study will be conducted according to the appropriate regulatory authority recommendations.

9.2 Schedule of Events

	Screening Visit	Inclusion Visit	Treatment Visit(s)	Post-Treatment Follow-up Period 1												Post-Treatment Follow-Up Period 2		
Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**12'[15] Phone visit**	**13/ EOS[1]**	**13'[16] Phone visit**	**14**	**15**	**16/ EOS[1]**
Day(s)	**-28 to -7**	**-2 or -1[2]**	**-1 and 0, or 0[3]**	**1**	**14 (±2D)**	**28 (±3D)**	**56 (±6D)**	**84 (±9D)**	**168 (±17D)**	**252 (±17D)**	**365 (±30D)**	**548 (±30D)**	**30D before Visit 13**	**730 (±30D)**	**14D after Visit 13**	**1,095 (±30D)**	**1,460 (±30D)**	**1,825 (±60D)**
Weeks(s)	**-4 to -1**				**2**	**4**	**8**	**12**	**24**	**36**	**52**	**78**	**100**	**104**	**106**	**156**	**208**	**260**
Month(s)						**1**	**2**	**3**	**6**	**9**	**12**	**18**	**23**	**24**	**24.5**	**36**	**48**	**60**
Year(s)											**1**	**1.5**	**1.92**	**2**	**2.04**	**3**	**4**	**5**
Signed Informed Consent - First Period	X																	
Signed Informed Consent - Second Period														X				
Selection/non-selection criteria	X																	
Inclusion/exclusion criteria		X																
Demographics[4]	X																	
LHON history	X																	
Relevant medical and surgical history	X																	
Prior medications	X																	
Physical Examination	X[5]													X		X	X	X
Vital signs	X[5]		X	X										X		X	X	X
12-Lead ECG	X[5]													X				X
Immune Modulating Regimen		X[6]																
Documentation of vision loss duration for each eye and determination of the first affected	X	X[7]																

and second affected/not yet affected eye																	
Determination of best- and worst-seeing eyes		X															
Laboratory Assessments																	
ND4 (G11778A) genotyping[8]	X	Review Lab Results to Confirm Eligibility															
Hematology / serum chemistry[9]	X												X		X	X	X
HIV testing[9]	X																
Serum pregnancy test[9]	X																
Urine pregnancy test[10]		X															
Humoral immune response to AAV2[11]	X			X	X	X	X	X		X	X		X				
Cellular immune response to AAV2[11]	X			X	X	X	X	X		X	X		X				
Blood for bio-dissemination[11]	X			X	X												
Quality of Life Assessments																	
VFQ-25[12]		X						X		X	X		X		X	X	X
SF-36v2[12]		X						X		X	X		X		X	X	X
Ocular & Vision Assessments																	
Refraction for BCVA	X	X		X	X	X	X	X	X	X	X	X		X		X	X	X
Visual acuity	X	X		X	X	X	X	X	X	X	X	X		X		X	X	X
Pelli-Robson contrast sensitivity	X	X		X	X	X	X	X	X	X	X	X		X		X	X	X
Humphrey Visual Field 30-2	X	X		X	X	X	X	X	X	X	X	X		X		X	X	X
Goldmann applanation tonometry for IOP[13]	X	X	X	X	X	X	X	X	X	X	X	X		X		X	X	X
Slit lamp examination (pre- and post-pupil dilation)	X	X	X	X	X	X	X	X	X	X	X	X		X		X	X	X

Color fundus photos		X		X	X	X	X	X	X	X	X	X		X		X	X	X
Spectral-Domain Optical Coherence Tomography	X	X		X	X	X	X	X	X	X	X	X		X		X	X	X
Fluorescein angiography[14]				X	X	X	X	X	X	X	X	X		X		X	X	X
IMPs																		
GS010/placebo intravitreal injection			X															
Safety Assessments																		
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X		X		X	X	X
Adverse and serious adverse events	X	X	X	X	X	X	X	X	X	X	X	X		X		X	X	X

Abbreviations: EOS = End of Study Visit; D = day; ECG = Electrocardiogram; AAV2 = Adeno-Associated Virus Vector serotype 2; *ND4* = NADH dehydrogenase 4; IOP = Intraocular pressure; BCVA = Best Corrected Visual Acuity; VFQ-25 = Visual Functioning Questionnaire-25; SF-36v2 = 36-Item Short Form Health Survey Version 2; IMP = Investigation Medicinal Product.

Footnotes:

1. Subjects who discontinue participation in the study prematurely (i.e. prior to completing Visit 13 or Visit 16) will complete an End of Study Visit, including all procedures listed for Visit 13 if subjects discontinue during the Post-Treatment Follow-Up Period 1 or Visit 16 if subjects discontinue during the Post-Treatment Follow-Up Period 2.
2. Inclusion Visit (Visit 2) may be performed on day -2 or day -1 in reference to Study Day 0, as determined by the treatment administration option selected (please see Section 9.1 and Table 1 for details).
3. Treatment Visit(s) (Visit 3) may be performed on Day -1 and Study Day 0, or only on Study Day 0 as determined by the treatment administration option selected (please see Section 9.1 and Table 1 for details). Study Day 0 is the day that treatment administration is completed to both eyes; in the setting of treatment administration on consecutive days, this will be the second treatment day. All timing of visits and visit windows are in reference to this Study Day 0.
4. Including tobacco use and alcohol consumption.
5. These examinations will serve screening purposes and serve as baseline values for comparison to post-treatment values.
6. The immune modulating regimen will commence 2-days prior to the initial treatment administration to any eye of the subject and will be completed after a 28-day immune modulating regimen.
7. At the Inclusion Visit (Visit 2) only new onset vision loss occurring since Visit 1 in eyes previously unaffected by clinically manifested visual acuity loss will be recorded.

8. All subjects are required to have a blood sample for *ND4* genotyping sent to the study's central laboratory. Eligibility for selection/inclusion may be determined with a reliable genotyping result previously obtained at a non-study laboratory confirming the G11778A *ND4* mitochondrial mutation and the absence of the *ND1* and *ND6* mitochondrial mutations.
9. All subjects are required to have blood samples for hematology, serum chemistries, HIV testing and serum pregnancy test (for women of childbearing potential) sent to the study's central laboratory. Eligibility for selection/inclusion may be determined with recently performed, reliable test results obtained at a local, non-study laboratory.
10. Highly sensitive urine pregnancy test. To be performed only if applicable per local regulatory requirement. Required only if serum pregnancy test obtained at Visit 1 was performed more than 7 days prior to Visit 3. Must be analyzed at local study site laboratory.
11. Baseline, pre-treatment blood samples for humoral and cellular immune responses and bio-dissemination must be obtained prior to initiating the immune modulating regimen.
12. QoL questionnaires must be performed prior to any vision and ocular assessments.
13. Goldmann applanation tonometry for IOP measurement must be performed pre-pupil dilation except at post-pupil dilation timepoints at Treatment Visit(s) (Visit(s) 3).
14. Fluorescein angiography may be performed per investigator discretion for significant vitreous inflammation or posterior uveitis, or as deemed necessary per the investigator for the management of the subject's clinical condition.
15. If a subject did not receive the ICF related to the Second Period of the study or Post-Treatment Follow-up Period 2 during Visit 12, a telephone visit will be set up approximately 1 month before Visit 13.
16. If a subject did not receive the ICF related to the Second Period of the study or Post-Treatment Follow-up Period 2 during Visit 13, a telephone visit will be set up approximately 2 weeks after Visit 13.

10. Study Population

The study population will include subjects with vision loss from LHON due to the G11778A point mutation in the mitochondrial *ND4* gene. Subjects with vision loss of up to 1 year in duration will be included.

10.1 Number of Subjects

Details of the estimations for the sample size calculation are provided in Section 16. Briefly, considering the study design with two treatment arms and a Standard Deviation (SD) of 0.50 for the change from baseline of the LogMAR visual acuity in *ND4* LHON subjects, to show a clinically significant difference of 0.3 LogMAR (equivalent to 15 ETDRS letters) with a study power of 80%, approximately 45 treated and evaluable subjects are needed per treatment arm and approximately 90 treated and evaluable subjects are needed for the study in total.

It is estimated that the screen failure rate for the intended study population will be approximately 23%. Therefore, to reach approximately 90 treated and evaluable subjects for the study, approximately 117 subjects will be screened.

Subjects who do not meet selection and non-selection criteria may be re-screened under Sponsor approval.

It is expected that the rate of dropout from the study prior to the primary timepoint (i.e. Year 1.5) will be low, estimated to be less than 5%. Depending on the number of subjects who terminate early, additional subjects may be enrolled through the end of the recruitment period.

10.2 Selection for the Study

10.2.1 Selection Criteria

Subjects must meet all the following criteria at Screening Visit (Visit 1) to be eligible for inclusion.

1. Age 15 years or older on the date of signed informed consent.
2. Clinically manifested vision loss due to *ND4* LHON, to any extent, in at least one eye.
3. Vision loss duration of ≤ 365 days (i.e. ≤ 1 year) in each affected eye at Inclusion Visit (Visit 2).
4. Female subjects (if of childbearing potential) must agree to use effective methods of birth control for up to 6 months after Treatment Visit(s) 3 and male subjects must agree to use condoms for up to 6 months after Treatment Visit(s) 3.
5. Ability to obtain adequate pupillary dilation to permit thorough ocular examination and visual testing.
6. Subject – and parent/legal guardian if the subject is under 18 years of age – has provided signed, written informed consent.

10.2.2 Non-Selection Criteria

Subjects meeting any of the following criteria at Screening Visit 1 will be excluded from eligibility.

1. Any known allergy or hypersensitivity to GS010 or its constituents.

2. Contraindication to intravitreal injection in any eye.
3. Intravitreal drug delivery to any eye within 30 days prior to the Screening Visit (Visit 1).
4. Previous vitrectomy in either eye.
5. Narrow angle in any eye contra-indicating pupillary dilation.
6. Presence of disorders or diseases of the eye or adnexa, excluding LHON, which may interfere with visual or ocular assessments, including SD-OCT, during the study period.
7. Presence of known/documented mutations, other than the G11778A *ND4* LHON-causing mutation, which are known to cause pathology of the optic nerve, retina or afferent visual system.
8. Presence of systemic or ocular/vision diseases, disorders or pathologies, other than LHON, known to cause or be associated with vision loss, or whose associated treatment(s) or therapy(ies) is/are known to cause or be associated with vision loss.
9. Presence of optic neuropathy from any cause except LHON.
10. Presence of illness or disease that, in the opinion of the Investigator, include symptoms and/or the associated treatments that can alter visual function, for instance cancers or pathology of the central nervous system, including Multiple Sclerosis (diagnosis of Multiple Sclerosis must be based on the 2010 Revisions to the McDonald Criteria [Polman 2011]).
11. History of recurrent uveitis (idiopathic or immune-related) or active ocular inflammation.
12. Participation in another clinical trial and receiving an IMP within 90 days prior to the Screening Visit (Visit 1). Exception: subjects remain eligible if they completed a clinical trial with idebenone as an IMP less than 90 days prior to Visit 1 AND completely discontinued idebenone at least 7 days prior to Visit 2.
13. Previous treatment with ocular gene therapy in either eye.
14. Subjects refusing to discontinue idebenone.
15. Subjects who have undergone ocular surgery of clinical relevance (per Investigator assessment) within 90 days preceding the Screening Visit (Visit 1).
16. Female Subjects who are, or who intend to breast feed during the initial six months' post-administration of GS010.
17. Subjects who unable to tolerate (e.g. the immune modulating regimen) or unable or unwilling to comply with all the protocol requirements.

10.2.3 Inclusion Criteria

Subjects must meet all the following criteria at Inclusion Visit (Visit 2) to be eligible for inclusion.

1. Vision loss duration of ≤ 365 days (i.e. ≤ 1 year) in each affected eye at Inclusion Visit (Visit 2).
2. Each eye of the subject must maintain at least Hand Motion (HM) visual acuity, as defined by the study's SOP for visual acuity testing.
3. Documented results of genotyping showing the presence of the G11778A mutation in the *ND4* gene and the absence of the other primary LHON-associated mutations (*ND1* or *ND6)* in the subject's mitochondrial DNA.
4. Have a negative test for infection with human immunodeficiency virus (HIV).

5. Female subjects of childbearing potential must have a negative pregnancy test for (a woman who is two years post-menopausal or surgically sterile is not considered to be of childbearing potential).
6. Review of all selection criteria to ensure continued compliance.

10.2.4 Exclusion Criteria

Subjects meeting any of the following criteria at Inclusion Visit 2 will be excluded from eligibility.

1. Light Perception (LP) or No Light Perception (NLP) visual acuity in any eye, as defined by the study's standard operating procedure (SOP) for visual acuity testing.
2. Subjects taking idebenone who have not completely discontinued the idebenone at least 7 days prior to Visit 2. If the subject has not discontinued idebenone at least 7 days prior to Visit 2, Visit 2 may be delayed until the 7-day period is complete as long as the study visit windows are adhered to.
3. Presence of active infectious conjunctivitis, keratitis, scleritis or endophthalmitis in either eye.
4. Presence of alcoholism, alcohol dependence, or alcohol or drug abuse (excluding nicotine).
5. Presence of systemic illness or medically significant abnormal laboratory values that are deemed by the Investigator to preclude the subject's safe participation in the study.
6. Any medical or psychological condition that, in the opinion of the Investigator, may lead to non-tolerance of the treatment regimen, may compromise the safe participation of the subject in the study or would preclude compliance with the study protocol or ability of the subject to successfully complete the study.
7. Any non-selection criteria which may have appeared after the screening visit.

10.2.5 Deviation from Criteria for Selection

No protocol deviation related to study inclusion or exclusion criteria, conduct of the trial, subject management, or subject assessment will be allowed prospectively.

11. Study Conduct

Note: Study Day 0 is the day that treatment administration is completed to both eyes; in the setting of treatment administration on consecutive days, this will be the second treatment day. All timing of visits and visit windows are in reference to this Study Day 0.

11.1 Study Procedures by Visit

11.1.1 Screening Visit (Visit 1)

Screening Visit 1 will be conducted from 28 to 7 days prior to Study Day 0. The following procedures will be performed at Visit 1:

- Informed consent of First Period read, understood, and signed by the subject. If the subject is unable to read the informed consent document, presence of an impartial witness is required to confirm that the contents of the document were explained to the subject. The impartial witness must sign the informed consent form (ICF). The subject must always be asked to sign or mark the ICF regardless of their visual ability. If the subject is under the legal age of consent, the potential subject's parent or legal guardian will be given an ICF for review, and the minor subject will sign a pediatric assent form, according to applicable local regulation.
- Assess subject's eligibility for the study with the selection and non-selection criteria. If the subject fails to comply with any of these criteria, the subject will not be selected for study participation and no further assessments will be conducted.
- Collection of subject demographic information, including gender, date of birth and age, tobacco use (e.g. cigarette smoking) and alcohol consumption, relevant medical and surgical history, including history related to symptoms and diagnosis of LHON, and prior relevant medications.
- Documentation of vision loss duration for each eye and determination of the first affected eye and the second affected/not yet affected eye.
- Assessment of physical examination, including general appearance, skin, head, eyes, ears, nose, throat, neck, thyroid, chest/lungs, heart, abdomen, lymph nodes, extremities, height, and body weight.
- Assessment of vital signs including blood pressure (BP), pulse rate, and temperature.
- 12-lead ECG.
- Blood draw for genotyping to confirm the presence of the G11778A mutation in the mitochondrial *ND4* gene. All subjects are required to have a blood sample for genotyping sent to the study's central laboratory. Eligibility for selection/inclusion may be determined with a prior, reliable genotyping result obtained at a non-study laboratory, confirming the G11778A *ND4* mitochondrial mutation and the absence of the *ND1* and *ND6* mitochondrial mutations.
- Blood draws for hematology, serum chemistries, HIV testing and pregnancy testing for female subjects of childbearing potential. All subjects are required to have blood samples for hematology, serum chemistries, HIV testing and serum pregnancy test (for female subjects of childbearing potential) sent to the study's central laboratory. Eligibility for

selection/inclusion may be determined with recently performed, reliable test results obtained at a local, non-study laboratory.

- Blood draw for humoral immune response to AAV2, obtained prior to initiation of the immune modulating regimen.
- Blood draw for cellular immune response to AAV2, obtained prior to initiation of the immune modulating regimen.
- Blood draw for bio-dissemination, obtained prior to initiation of the immune modulating regimen.
- Refraction for BCVA, performed pdrior to pupil dilation.
- Visual acuity assessment performed prior to pupil dilation.
- Pelli-Robson contrast sensitivity test performed prior to pupil dilation.
- HVF 30-2, performed prior to pupil dilation.
- Goldmann applanation tonometry for IOP measurement, performed prior to pupil dilation.
- Slit lamp examination (SLE) performed pre- and post-pupil dilation.
- SD-OCT, performed after pupil dilation.
- Recording of any AE or SAE.
- Recording of any concomitant medications and supplements.
- The immune modulating regimen will be initiated after conducting the Screening Visit (Visit 1) and prior to conducting the Inclusion Visit (Visit 2).

11.1.2 Inclusion Visit (Visit 2)

Inclusion Visit (Visit 2) will be conducted 2 days prior to Study Day 0, or 1 day prior to Study Day 0, as determined by the treatment administration option selected. The following procedures will be performed at Visit 2:

- Assess subject's eligibility for to the study with the inclusion and exclusion criteria. If the subject fails to comply with any of these criteria, the subject will be excluded from the study and no further assessments will be conducted.
- Record the occurrence of new onset vision loss occurring since Visit 1 in eyes previously unaffected by clinically manifested vision loss with documentation of the date of onset of vision loss and the vision loss duration.
- Determination and documentation of the best-seeing and worst-seeing eye of the subject.
- Review of laboratory assessment results obtained at Visit 1 to confirm subject's eligibility for inclusion.
- Highly sensitive urine pregnancy test obtained only if applicable per local regulatory requirement.
- Assessment of VFQ-25 QoL scale. This must be performed prior to any vision or ocular assessments.
- Assessment of SF-36v2 QoL scale. This must be performed prior to any vision or ocular assessments.
- Refraction for BCVA, performed prior to pupil dilation.
- Visual acuity assessment performed prior to pupil dilation.
- Pelli-Robson contrast sensitivity test performed prior to pupil dilation.

- HVF 30-2, performed prior to pupil dilation.
- Goldmann applanation tonometry for IOP measurement, performed prior to pupil dilation.
- SLE, performed pre- and post-pupil dilation.
- SD-OCT performed after pupil dilation.
- Color fundus photo performed after pupil dilation.
- Recording any AE or SAE.
- Recording of any changes and additions to concomitant medications and any changes in concomitant medication dose or regimen, including supplements.

11.1.3 Treatment Visit (Visit 3)

Treatment Visit(s) 3 may be conducted in one day or in two consecutive days (i.e. Study Day 0, or day -1 and Study Day 0). The initial treatment administration must be conducted the day following the Inclusion Visit (Visit 2). When both eyes are treated the same day, treatment must be performed the day after Inclusion Visit (Visit 2). The following procedures will be performed at the treatment administration visit(s):

- Assessment of vital signs including BP, pulse rate, and temperature, per the Intravitreal GS010/Placebo Injection Guide.
- Goldmann applanation tonometry for IOP measurement, per the Intravitreal GS010/Placebo Injection Guide.
- SLE, performed pre- and post-pupil dilation per the Intravitreal GS010/Placebo Injection Guide.
- Administration of GS010 and/or placebo via intravitreal injection, according to the randomized treatment allocation, per the Intravitreal GS010/Placebo Injection Guide.
- Recording of any changes and additions to concomitant medications and any changes in concomitant medication dose or regimen, including supplements, pre- and post-IMP/placebo administration.
- Recording any AE or SAE that has occurred, pre- and post-IMP/placebo administration.

11.1.4 Post-Treatment Follow-Up Period 1 and Period 2: Visits 4 through 16

Follow-up visits (Visits 4 through Visit 16) will be conducted post-treatment administration. During the Post-Treatment Follow-Up Period 1, ten follow-up visits, Visits 4 through 13 with 2 optional telephone visits, then in the Post-Treatment Follow-Up Period 2, 3 additional follow-up visits, Visit 14 through 16, are scheduled to be conducted at the following time points and with the following assessments:

11.1.4.1 Visit 4 – Day 1

Visit 4 will be conducted one day after Study Day 0, i.e. one day after completion of the treatment administration to both eyes of a subject. The following procedures will be conducted at Visit 4:

- Assessment of vital signs including BP, pulse rate, and temperature.
- Refraction for BCVA, performed prior to pupil dilation.
- Visual acuity assessment performed prior to pupil dilation.

- Pelli-Robson contrast sensitivity test performed prior to pupil dilation.
- HVF 30-2, performed prior to pupil dilation.
- Goldmann applanation tonometry for IOP measurement, performed prior to pupil dilation.
- SLE, performed pre- and post-pupil dilation.
- SD-OCT performed after pupil dilation.
- Color fundus photo performed after pupil dilation.
- Fluorescein angiography, performed post-pupil dilation, may be obtained per Investigator discretion for significant vitreous inflammation or posterior uveitis or as deemed necessary per the investigator for management of the subject's clinical condition.
- Recording of any AE or SAE.
- Recording of any changes and additions to concomitant medications and any changes in concomitant medication dose or regimen, including supplements.

11.1.4.2 Visit 5 – Week 2

Visit 5 will be conducted 14 ± 2 days after Study Day 0. The following procedures will be conducted at Visit 5:

- Blood draw for humoral immune response to AAV2.
- Blood draw for cellular immune response to AAV2.
- Blood draw for bio-dissemination.
- Refraction for BCVA, performed prior to pupil dilation.
- Visual acuity assessment performed prior to pupil dilation.
- Pelli-Robson contrast sensitivity test performed prior to pupil dilation.
- HVF 30-2, performed prior to pupil dilation.
- Goldmann applanation tonometry for IOP measurement, performed prior to pupil dilation.
- SLE, performed pre- and post-pupil dilation.
- SD-OCT performed after pupil dilation.
- Color fundus photo performed after pupil dilation.
- Fluorescein angiography, performed post-pupil dilation, may be obtained per investigator discretion for significant vitreous inflammation or posterior uveitis or as deemed necessary per the investigator for management of the subject's clinical condition.
- Recording of any AE or SAE.
- Recording of any changes and additions to concomitant medications and any changes in concomitant medication dose or regimen, including supplements.

11.1.4.3 Visit 6 – Week 4 / Month 1

Visit 6 will be conducted 28 ± 3 days after Study Day 0. The following procedures will be conducted at Visit 6:

- Blood draw for humoral immune response to AAV2.
- Blood draw for cellular immune response to AAV2.
- Blood draw for bio-dissemination.
- Refraction for BCVA, performed prior to pupil dilation.

- Visual acuity assessment performed prior to pupil dilation.
- Pelli-Robson contrast sensitivity test performed prior to pupil dilation.
- HVF 30-2, performed prior to pupil dilation.
- Goldmann applanation tonometry for IOP measurement, performed prior to pupil dilation.
- SLE, performed pre- and post-pupil dilation.
- SD-OCT performed after pupil dilation.
- Color fundus photo performed after pupil dilation.
- Fluorescein angiography, performed post-pupil dilation, may be obtained per investigator discretion for significant vitreous inflammation or posterior uveitis or as deemed necessary per the investigator for management of the subject's clinical condition.
- Recording of any AE or SAE.
- Recording of any changes and additions to concomitant medications and any changes in concomitant medication dose or regimen, including supplements.

11.1.4.4 Visit 7 – Week 8 / Month 2

Visit 7 will be conducted 56 ± 6 days after Study Day 0. The following procedures will be conducted at Visit 7:

- Blood draw for humoral immune response to AAV2.
- Blood draw for cellular immune response to AAV2.
- Refraction for BCVA, performed prior to pupil dilation.
- Visual acuity assessment performed prior to pupil dilation.
- Pelli-Robson contrast sensitivity test performed prior to pupil dilation.
- HVF 30-2, performed prior to pupil dilation.
- Goldmann applanation tonometry for IOP measurement, performed prior to pupil dilation.
- SLE, performed pre- and post-pupil dilation.
- SD-OCT performed after pupil dilation.
- Color fundus photo performed after pupil dilation.
- Fluorescein angiography, performed post-pupil dilation, may be obtained per investigator discretion for significant vitreous inflammation or posterior uveitis or as deemed necessary per the investigator for management of the subject's clinical condition.
- Recording of any AE or SAE.
- Recording of any changes and additions to concomitant medications and any changes in concomitant medication dose or regimen, including supplements.

11.1.4.5 Visit 8 – Week 12 / Month 3

Visit 8 will be conducted 84 ± 9 days after Study Day 0. The following procedures will be conducted at Visit 8:

- Blood draw for humoral immune response to AAV2.
- Blood draw for cellular immune response to AAV2.
- Refraction for BCVA, performed prior to pupil dilation.
- Visual acuity assessment performed prior to pupil dilation.

- Pelli-Robson contrast sensitivity test performed prior to pupil dilation.
- HVF 30-2, performed prior to pupil dilation.
- Goldmann applanation tonometry for IOP measurement, performed prior to pupil dilation.
- SLE, performed pre- and post-pupil dilation.
- SD-OCT performed after pupil dilation.
- Color fundus photo performed after pupil dilation.
- Fluorescein angiography, performed post-pupil dilation, may be obtained per investigator discretion for significant vitreous inflammation or posterior uveitis or as deemed necessary per the investigator for management of the subject's clinical condition.
- Recording of any AE or SAE.
- Recording of any changes and additions to concomitant medications and any changes in concomitant medication dose or regimen, including supplements.

11.1.4.6 Visit 9 – Week 24 / Month 6

Visit 9 will be conducted 168 ± 17 days after Study Day 0. The following procedures will be conducted at Visit 9:

- Blood draw for humoral immune response to AAV2.
- Blood draw for cellular immune response to AAV2.
- Assessment of VFQ-25 QoL scale. This must be performed prior to any vision or ocular assessments.
- Assessment of SF-36v2 QoL scale. This must be performed prior to any vision or ocular assessments.
- Refraction for BCVA, performed prior to pupil dilation.
- Visual acuity assessment performed prior to pupil dilation.
- Pelli-Robson contrast sensitivity test performed prior to pupil dilation.
- HVF 30-2, performed prior to pupil dilation.
- Goldmann applanation tonometry for IOP measurement, performed prior to pupil dilation.
- SLE, performed pre- and post-pupil dilation.
- SD-OCT performed after pupil dilation.
- Color fundus photo performed after pupil dilation.
- Fluorescein angiography, performed post-pupil dilation, may be obtained per investigator discretion for significant vitreous inflammation or posterior uveitis or as deemed necessary per the investigator for management of the subject's clinical condition.
- Recording of any AE or SAE.
- Recording of any changes and additions to concomitant medications and any changes in concomitant medication dose or regimen, including supplements.

11.1.4.7 Visit 10 – Week 36 / Month 9

Visit 10 will be conducted 252 ± 17 days after Study Day 0. The following procedures will be conducted at Visit 10:

- Refraction for BCVA, performed prior to pupil dilation.

- Visual acuity assessment performed prior to pupil dilation.
- Pelli-Robson contrast sensitivity test performed prior to pupil dilation.
- HVF 30-2, performed prior to pupil dilation.
- Goldmann applanation tonometry for IOP measurement, performed prior to pupil dilation.
- SLE, performed pre- and post-pupil dilation.
- SD-OCT performed after pupil dilation.
- Color fundus photo performed after pupil dilation.
- Fluorescein angiography, performed post-pupil dilation, may be obtained per investigator discretion for significant vitreous inflammation or posterior uveitis or as deemed necessary per the investigator for management of the subject's clinical condition.
- Recording of any AE or SAE.
- Recording of any changes and additions to concomitant medications and any changes in concomitant medication dose or regimen, including supplements.

11.1.4.8 Visit 11 – Week 52 / Year 1

Visit 11 will be conducted 365 ± 30 days after Study Day 0. The following procedures will be conducted at Visit 11:

- Blood draw for humoral immune response to AAV2.
- Blood draw for cellular immune response to AAV2.
- Assessment of VFQ-25 QoL scale. This must be performed prior to any vision or ocular assessments.
- Assessment of SF-36v2 QoL scale. This must be performed prior to any vision or ocular assessments.
- Refraction for BCVA, performed prior to pupil dilation.
- Visual acuity assessment performed prior to pupil dilation.
- Pelli-Robson contrast sensitivity test performed prior to pupil dilation.
- HVF 30-2, performed prior to pupil dilation.
- Goldmann applanation tonometry for IOP measurement, performed prior to pupil dilation.
- SLE, performed pre- and post-pupil dilation.
- SD-OCT performed after pupil dilation.
- Color fundus photo performed after pupil dilation.
- Fluorescein angiography, performed post-pupil dilation, may be obtained per investigator discretion for significant vitreous inflammation or posterior uveitis or as deemed necessary per the investigator for management of the subject's clinical condition.
- Recording of any AE or SAE.
- Recording of any changes and additions to concomitant medications and any changes in concomitant medication dose or regimen, including supplements.

11.1.4.9 Visit 12 – Week 78 / Year 1.5

Visit 12 will be conducted 548 ± 30 days after Study Day 0. The following procedures will be conducted at Visit 12:

- Informed consent of the Second Period of the study or Post-Treatment Follow-up Period 2 provided.
- Blood draw for humoral immune response to AAV2.
- Blood draw for cellular immune response to AAV2.
- Assessment of VFQ-25 QoL scale. This must be performed prior to any vision or ocular assessments.
- Assessment of SF-36v2 QoL scale. This must be performed prior to any vision or ocular assessments.
- Refraction for BCVA, performed prior to pupil dilation.
- Visual acuity assessment performed prior to pupil dilation.
- Pelli-Robson contrast sensitivity test performed prior to pupil dilation.
- HVF 30-2, performed prior to pupil dilation.
- Goldmann applanation tonometry for IOP measurement, performed prior to pupil dilation.
- SLE, performed pre- and post-pupil dilation.
- SD-OCT performed after pupil dilation.
- Color fundus photo performed after pupil dilation.
- Fluorescein angiography, performed post-pupil dilation, may be obtained per investigator discretion for significant vitreous inflammation or posterior uveitis or as deemed necessary per the investigator for management of the subject's clinical condition.
- Recording of any AE or SAE.
- Recording of any changes and additions to concomitant medications and any changes in concomitant medication dose or regimen, including supplements.

Subjects who were not provided with the ICF of the Second Period or Post-Treatment Follow-up Period 2 during Visit 12 will have a telephone visit scheduled approximately 1 month before Visit 13 to introduce the long-term follow-up period of the REFLECT study.

11.1.4.10 Visit 13 – Week 104 / Year 2 / End of Study Visit

Visit 13 will be conducted 730 ± 30 days after Study Day 0. The procedures listed below will also be performed in the case of premature withdrawal during the Post-Treatment Follow-Up Period 1 of the study (i.e. prior to Visit 13 or if the subject refuses to take part in the Post-Treatment Follow-up Period 2). The following procedures will be conducted at Visit 13 or End of Study Visit:

- Informed consent of Second Period or Post-Treatment Follow-up Period 2 read, understood, and signed by the subject. If the subject is unable to read the informed consent document, presence of an impartial witness is required to confirm that the contents of the document were explained to the subject. The impartial witness must sign the informed consent form (ICF). The subject must always be asked to sign or mark the ICF regardless of their visual ability. If the subject is under the legal age of consent, the potential subject's parent or legal guardian will be given an ICF for review, and the minor subject will sign a pediatric assent form, according to applicable local regulation.
- Assessment of physical examination, including general appearance, skin, head, eyes, ears, nose, throat, neck, thyroid, chest/lungs, heart, abdomen, lymph nodes, extremities, height, and body weight.

- Assessment of vital signs including BP, pulse rate, and temperature.
- 12-lead ECG.
- Blood draws for hematology and serum chemistries.
- Blood draw for humoral immune response to AAV2.
- Blood draw for cellular immune response to AAV2.
- Assessment of VFQ-25 QoL scale. This must be performed prior to any vision or ocular assessments.
- Assessment of SF-36v2 QoL scale. This must be performed prior to any vision or ocular assessments.
- Refraction for BCVA, performed prior to pupil dilation.
- Visual acuity assessment performed prior to pupil dilation.
- Pelli-Robson contrast sensitivity test performed prior to pupil dilation.
- HVF 30-2, performed prior to pupil dilation.
- Goldmann applanation tonometry for IOP measurement, performed prior to pupil dilation.
- SLE, performed pre- and post-pupil dilation.
- SD-OCT performed after pupil dilation.
- Color fundus photo performed after pupil dilation.
- Fluorescein angiography, performed post-pupil dilation, may be obtained per investigator discretion for significant vitreous inflammation or posterior uveitis or as deemed necessary per the investigator for management of the subject's clinical condition.
- Recording of any AE or SAE.
- Recording of any changes and additions to concomitant medications and any changes in concomitant medication dose or regimen, including supplements.

Subjects who were not provided with the ICF of the Post-Treatment Follow-up Period 2 of the study during Visit 13 will have a telephone visit scheduled approximately 2 weeks after Visit 13 to introduce the long-term follow-up period of the REFLECT study.

11.1.4.11 Visit 14 – Week 156 / Year 3

Visit 14 will be conducted 1,095 ± 30 days after Study Day 0. The following procedures will be conducted at Visit 14:

- Assessment of physical examination, including general appearance, skin, head, eyes, ears, nose, throat, neck, thyroid, chest/lungs, heart, abdomen, lymph nodes, extremities, height, and body weight.
- Assessment of vital signs including BP, pulse rate, and temperature.
- Blood draws for hematology and serum chemistries.
- Assessment of VFQ-25 QoL scale. This must be performed prior to any vision or ocular assessments.
- Assessment of SF-36v2 QoL scale. This must be performed prior to any vision or ocular assessments.
- Refraction for BCVA, performed prior to pupil dilation.
- Visual acuity assessment performed prior to pupil dilation.

- Pelli-Robson contrast sensitivity test performed prior to pupil dilation.
- HVF 30-2, performed prior to pupil dilation.
- Goldmann applanation tonometry for IOP measurement, performed prior to pupil dilation.
- SLE, performed pre- and post-pupil dilation.
- SD-OCT performed after pupil dilation.
- Color fundus photo performed after pupil dilation.
- Fluorescein angiography, performed post-pupil dilation, may be obtained per investigator discretion for significant vitreous inflammation or posterior uveitis or as deemed necessary per the investigator for management of the subject's clinical condition.
- Recording of any AE or SAE.
- Recording of any changes and additions to concomitant medications and any changes in concomitant medication dose or regimen, including supplements.

11.1.4.12 Visit 15 – Week 208 / Year 4

Visit 15 will be conducted 1,460 ± 30 days after Study Day 0. The following procedures will be conducted at Visit 15:

- Assessment of physical examination, including general appearance, skin, head, eyes, ears, nose, throat, neck, thyroid, chest/lungs, heart, abdomen, lymph nodes, extremities, height, and body weight.
- Assessment of vital signs including BP, pulse rate, and temperature.
- Blood draws for hematology and serum chemistries.
- Assessment of VFQ-25 QoL scale. This must be performed prior to any vision or ocular assessments.
- Assessment of SF-36v2 QoL scale. This must be performed prior to any vision or ocular assessments.
- Refraction for BCVA, performed prior to pupil dilation.
- Visual acuity assessment performed prior to pupil dilation.
- Pelli-Robson contrast sensitivity test performed prior to pupil dilation.
- HVF 30-2, performed prior to pupil dilation.
- Goldmann applanation tonometry for IOP measurement, performed prior to pupil dilation.
- SLE, performed pre- and post-pupil dilation.
- SD-OCT performed after pupil dilation.
- Color fundus photo performed after pupil dilation.
- Fluorescein angiography, performed post-pupil dilation, may be obtained per investigator discretion for significant vitreous inflammation or posterior uveitis or as deemed necessary per the investigator for management of the subject's clinical condition.
- Recording of any AE or SAE.
- Recording of any changes and additions to concomitant medications and any changes in concomitant medication dose or regimen, including supplements.

11.1.4.13 Visit 16 – Week 260 / Year 5 / End of Study Visit

Visit 16 will be conducted 1,825 ± 60 days after Study Day 0. The procedures listed below will also be performed in the case of premature withdrawal during the Post-Treatment Follow-Up Period 2 (i.e. prior to Visit 16). The following procedures will be conducted at Visit 16 or End of Study Visit:

- Assessment of physical examination, including general appearance, skin, head, eyes, ears, nose, throat, neck, thyroid, chest/lungs, heart, abdomen, lymph nodes, extremities, height, and body weight.
- Assessment of vital signs including BP, pulse rate, and temperature.
- 12-lead ECG.
- Blood draws for hematology and serum chemistries.
- Assessment of VFQ-25 QoL scale. This must be performed prior to any vision or ocular assessments.
- Assessment of SF-36v2 QoL scale. This must be performed prior to any vision or ocular assessments.
- Refraction for BCVA, performed prior to pupil dilation.
- Visual acuity assessment performed prior to pupil dilation.
- Pelli-Robson contrast sensitivity test performed prior to pupil dilation.
- HVF 30-2, performed prior to pupil dilation.
- Goldmann applanation tonometry for IOP measurement, performed prior to pupil dilation.
- SLE, performed pre- and post-pupil dilation.
- SD-OCT performed after pupil dilation.
- Color fundus photo performed after pupil dilation.
- Fluorescein angiography, performed post-pupil dilation, may be obtained per investigator discretion for significant vitreous inflammation or posterior uveitis or as deemed necessary per the investigator for management of the subject's clinical condition.
- Recording of any AE or SAE.
- Recording of any changes and additions to concomitant medications and any changes in concomitant medication dose or regimen, including supplements.

11.2 Premature Discontinuation

Subjects who discontinue participation in the study (i.e. prior to completing Visit 13 or Visit 16) will complete an End of Study Visit, including all procedures listed for Visit 13 if discontinuation occurs during the Post-Treatment Follow-Up Period 1 or for Visit 16 if discontinuation occurs during the Post-Treatment Follow-Up Period 2.

A subject will be withdrawn from the study under any of the following circumstances:

- Subject's request at any time for any reason.
- Physician's determination that subject's further participation in the protocol is not in the subject's best interest.
- The Sponsor's decision.
- Competent Authorities' decision.

For any discontinuation, the Investigator will obtain all the required details and document the date of discontinuation and the reason for discontinuation in the electronic Case Report Form (eCRF). All efforts should be made to obtain the Year 1.5 (Week 78) visual acuity assessment when possible.

If the reason for withdrawing from the study is an AE, the specific event must be recorded in the eCRF.

11.3 Unscheduled Visits

Unscheduled visits may be performed at any time during the study to assess or follow-up adverse events, at the subject's request, or at the request of the investigator. The date and reason for the unscheduled visit should be recorded in the source documentation.

If unscheduled visits occur, the Investigator must at least record the following in the subject's source document and eCRF:

- Any AE/SAE.
- Reason for unscheduled visit.
- Recording of any changes or additions to concomitant medications.
- Any clinical assessments, vision and non-vision related, performed during the unscheduled visit.

Unscheduled visits will not alter the timing of the study visit schedule or the assessments performed at any scheduled visit.

12. Description of Study Procedures

12.1 Informed Consent

12.1.1 First Period

Prior to entering the study, the Investigator must explain to each potential subject (and the potential subject's parent/legal guardian if the subject is under the legal age of consent) the nature of the study, its purpose, procedures, expected duration, alternative therapies available, and the benefits and risks involved in study participation.

If the subject is of the legal age of consent, they will be given the Institutional Review Board (IRB)/Independent Ethics Committee (IEC)-approved ICF to review and the opportunity to ask questions. The subject will be informed of their right to withdraw from the study at any time without prejudice. The subject should be able to answer simple questions about the study after the ICF has been reviewed and explained. After this explanation and before any study-specific procedures have been performed, the subject must voluntarily sign and date the ICF to indicate the desire to participate in the study. If the subject is unable to read the informed consent document, presence of an impartial witness is required to confirm the contents of the document were explained to the subject. The impartial witness must also sign the ICF. The subject must always be asked to sign or mark the form regardless of their vision. The Investigator must also sign and date the ICF.

The time (hour and minute) the consent is signed must also be recorded by the subject or legal guardian and the person providing consent to the subject. Prior to participation in the study, the subject will receive a copy of the signed and dated/timed consent form along with an emergency card with contact information for the Investigator and clinical facility staff in the event of a medical emergency during the trial.

If the subject is under the legal age of consent, the potential subject's parent or legal guardian will be given IRB/IEC-approved ICF to review and the potential subject will be given an IRB/IEC-approved pediatric assent form. Both the potential subject and their parent/legal guardian will be given the opportunity to ask questions. Both the potential subject and their parent/legal guardian will be informed of the subject's right to withdraw from the study at any time without prejudice. The potential subject and their parent/legal guardian should be able to answer simple questions about the study after the ICF and assent form have been reviewed and explained. After this explanation and before any study-specific procedures have been performed, the parent/legal guardian must voluntarily sign and date the ICF to indicate their informed consent for the subject to participate in the study. The subject must sign and date the pediatric assent form to indicate their desire to participate in the study. The Investigator must also sign and date both the ICF and the assent form. The time (hour and minute) the consent and assent are signed must also be recorded by the subject (on the assent form), the parent/legal guardian (on the ICF), and the person providing consent/assent (on both forms). Prior to participation in the study, the subject's parent/legal guardian will receive copies of the signed and dated consent and assent forms along with an emergency card with contact information for the Investigator and clinical facility staff in the event of a medical emergency during the trial.

After the subject has signed the ICF, or as applicable in the case of a subject being under the age of legal consent, after the subject has signed the pediatric assent form and the subject's parent/legal guardian has signed the ICF, study procedures and assessments as detailed in section 11 and in section 12, will be performed.

12.1.2 Second Period

At Visit 12, each subject will be invited to participate in the Second Period or Post-Treatment Follow-up Period 2 of the study. The subject will receive the new ICF related to the Post-Treatment Follow-up Period 2. The subject will be able to read and ask any question to the Investigator during Visit 12.

At Visit 13, if the subject decides to participate in the Post-Treatment Follow-up Period 2 and before any study-specific procedures have been performed, the subject must voluntarily sign and date the ICF to indicate the desire to continue participating in the Second period of the study.

If a subject did not receive the new ICF during Visit 12, a telephone visit will be set up approximately 1 month before Visit 13. The goal of this telephone visit will be to introduce the extension of the REFLECT study called Post-Treatment Follow-up Period 2. After this call, the new ICF related to the Post-Treatment Follow-up Period 2 will be sent to the subject within 5 working days approximately. During the time period between the telephone visit and Visit 13, the subject will be able to read and ask any questions to the Investigator by phone* or email. If the

subject decides to participate in the Post-Treatment Follow-up Period 2, the subject will provide the new ICF signed at Visit 13.

If a subject did not receive the new ICF during Visit 13, a telephone visit will be set up approximately 2 weeks after that visit to inform the subject of the possibility to participate in the Post-Treatment Follow-up Period 2 of the study. The goal of this telephone visit will be to present the new ICF for the Post-Treatment Follow-up Period 2. After this call, the subject will receive by mail the new ICF related to the Post-Treatment Follow-up Period 2. The subject will be able to read and ask any questions to the Investigator by phone*. If the subject decides to participate in the Post-Treatment Follow-up Period 2, the subject will provide the new ICF signed at the latest one month after the visit 13.

*The telephone number of the Investigator is mentioned on the patient card.

12.2 Demographics and General Clinical Evaluations

12.2.1 Demographics, Relevant Medical and Surgical, LHON History, and Prior Medications

The subject's demographics, including date of birth, age and gender, tobacco use and alcohol consumption, relevant medical and surgical history and prior medications will be recorded (according to national data collection regulations). History of and reasons for any hospital admissions (medical/surgical/psychiatric/other) in the past 3 months will also be recorded.

All prior medications including prescription and non-prescription medications, preparations and health and/or dietary supplements taken by the subject within the previous 30 days will also be recorded.

History pertaining to the diagnosis of LHON will be obtained. This will include (but is not limited to):

- Which eye has lost vision (right and/or left).
- Date (month, date, year) of onset of vision loss in the right eye (if applicable).
- Date (month, date, year) of onset of vision loss in the left eye (if applicable).

12.2.2 Physical Examination

Physical examination will include general appearance, skin, head, eyes, ears, nose, throat, neck, thyroid, chest/lungs, heart, abdomen, lymph nodes, extremities, height and body weight.

All abnormalities should be clearly documented.

12.2.3 Vital Signs

Vital signs will include BP (after at least 5 minutes sitting at rest), pulse rate, and temperature.

All abnormalities should be clearly documented.

12.2.4 12-Lead Electrocardiogram

Resting 12-lead ECG will be recorded at Visit 1 and at Visit 13/Visit 16/End of Study with the subject supine and at rest for at least 10 minutes prior to start. ECG should be collected prior to blood collection.

The ECGs will be reviewed at the site to assess any abnormalities. It is the responsibility of the Investigator or their medically qualified designee to review the results of all ECGs to assess for any abnormalities.

All abnormalities should be clearly documented.

12.3 Laboratory Evaluations

12.3.1 *ND4* (G11778A) Genotyping

All subjects are required to have a blood sample for *ND4* genotyping sent to the study's central laboratory at Screening Visit 1. Eligibility for selection/inclusion may be determined with a prior reliable genotyping result obtained at a non-study laboratory confirming the G11778A *ND4* mitochondrial mutation and the absence of the *ND1* and *ND6* mitochondrial LHON point mutations. The presence of the G11778A *ND4* mitochondrial DNA mutation must be documented by the completion of Inclusion Visit 2 and prior to randomization.

12.3.2 Hematology and Serum Chemistries

Blood samples will be collected for the following hematology and serum chemistries tests during the study per the study schedule:

- Hematology tests: Complete blood count including red blood cells, hemoglobin, hematocrit, white blood cells with differential, and platelets.
- Serum chemistry tests: Glucose, lipase, amylase, calcium, sodium, potassium, chloride, bicarbonate, blood urea nitrogen (BUN) and creatinine and the Liver Function Tests: AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), γGT (Gamma-glutamyl transpeptidase), total bilirubin, and albumin.

All subjects are required to provide a blood sample at Screening Visit 1 for hematology and serum chemistries. Eligibility for inclusion may be determined with recently performed, reliable, hematology and serum chemistries obtained at a local, non-study laboratory or reliable hematology and serum chemistries to be obtained at a local non-study laboratory. In all cases (i.e. use of local or central laboratory results for determination of inclusion eligibility) results must be available by Inclusion Visit 2 to be considered for eligibility for inclusion. At all subsequent visits hematology and serum chemistries will be performed only at the study's central laboratory only.

12.3.3 HIV Testing

Blood samples for HIV testing may be obtained for analysis in the study's central lab at the Screening Visit 1. However, eligibility for inclusion may be determined with a recently performed, reliable, HIV test obtained at a local, non-study laboratory, or a reliable HIV test to be obtained at

a local, non-study laboratory. In all cases the results of the HIV test must be available and documented by Visit 2. Subjects with positive tests for HIV will be excluded from study inclusion.

12.3.4 Serum Pregnancy Test

Blood samples for serum pregnancy test must be obtained for analysis in the study's central laboratory at Screening Visit 1 for female subjects of childbearing potential. However, eligibility for inclusion may be determined with recently performed, reliable, serum pregnancy test obtained at a local, non-study laboratory or a reliable serum pregnancy test to be obtained at a local non-study laboratory. In all cases the results of the serum pregnancy test must be available and documented by Visit 2. Subjects with positive serum pregnancy tests will be excluded from study inclusion

12.3.5 Highly Sensitive Urine Pregnancy Test

Urine sample for highly sensitive urine pregnancy test must be obtained only if applicable per local regulatory requirement. When applicable, this test must be obtained only if the serum pregnancy test obtained at Screening Visit (Visit 1) was performed more than 7 days prior to Treatment Visit (Visit 3, i.e. the first treatment administration). This test must be performed at the local study site laboratory and the results must be available to confirm eligibility.

12.3.6 Humoral Immune Response to AAV2

Blood samples for the humoral immune response to AAV2 will be obtained per the study schedule. All testing will be performed at the study's central laboratory.

Humoral immune response includes assessment of anti-AAV2 neutralizing antibodies, assessed via a cell-based seroneutralization assay.

12.3.7 Cellular Immune Response to AAV2

Blood samples for the cellular immune response to AAV2 will be obtained per the study schedule. All testing will be performed at the study's central laboratory.

Cellular immune response against AAV2 vector will be assessed by IFNγ ELIspot assay.

12.3.8 Bio-Dissemination

Blood samples for bio-dissemination of the AAV2 vector will be obtained per the study schedule. All testing will be performed at the study's central laboratory.

Presence of GS010 vector DNA in the blood will be assessed by determining the copy number of vector DNA with a quantitative polymerase chain reaction (qPCR) assay.

12.3.9 Sample Collection, Storage, Shipping

All blood sampling will be performed via venipuncture. Blood sample collection and processing procedures for the central study laboratory will be outlined in a separate reference manual to be provided to the site prior to the start of the study.

12.3.10 Laboratory Results Review

The Investigator or their designee will review the results of all laboratory results, except immune response and bio-dissemination results, in a timely manner. The investigator must provide comment on the laboratory results sheet for all abnormal values, identifying those that are abnormal and not clinically significant as well as those that are abnormal and clinically significant. The Investigator will also review the results of any tests conducted at local laboratories. Bio-dissemination and immune response laboratory results will not be reviewed by the investigator in an ongoing fashion; non-monitored immune response and bio-dissemination data of all subjects will be periodically reviewed by the DSMB and monitored results will be available for the 1-year post-treatment timepoint.

It is the Investigator's responsibility to review the results of laboratory tests as they become available. This review will be documented by the Investigator's dated signature on the laboratory report. For each abnormal laboratory test result, the Investigator needs to ascertain if this is a clinically significant abnormal change from baseline for the given individual subject.

12.4 Quality of Life Assessments

QoL questionnaires will be administered in paper version at the study site. An interviewer will administer the questionnaire to the subject. QoL questionnaires must be performed prior to any vision or ocular testing.

12.4.1.1 Visual Functioning Questionnaire-25

The VFQ-25 Questionnaire is a 25-item version of the 51-item National Eye Institute Visual Functioning Questionnaire. The VFQ-25 consists of a base set of 25 vision-targeted questions representing 11 vision-related constructs, plus an additional single-item general health rating question. All items are scored from 0 to 5 so that a higher number represents better functioning. To score the questionnaire, each item is converted to a 0-100 scale. Items within each subscale are averaged to create 12 subscale scores. The subscale scores (excluding the general health rating question) are then averaged to calculate the composite score (Mangione 2001).

12.4.1.2 36-Item Short Form Health Survey, Version 2

The SF-36 Questionnaire is a generic, subject-reported outcome instrument used to assess QoL. The SF-36 is a 36-question instrument, which assesses 8 health concepts: 1) limitations in physical activities because of health problems; 2) limitations in social activities because of physical or emotional problems; 3) limitations in usual role activities because of physical health problems; 4) bodily pain; 5) general mental health (psychological distress and well-being); 6) limitations in usual role activities because of emotional problems; 7) vitality (energy and fatigue); and 8) general health perceptions. The scale score of each domain is calculated based on the summed score across items included in the domain and is rescaled to 0 to 100 with higher scores indicating better health states (Ware 2007).

12.5 Ocular and Vision Assessments

Descriptions of the methods for ocular and vision testing are detailed in the SOP for Ocular and Vision Testing. Please refer to this SOP for complete details. A central ophthalmology reading center will perform quality control and analysis of ocular and vision assessments as detailed in the study-specific SOP for the central ophthalmology reading center.

12.5.1 Refraction for BCVA

Refraction will be performed per the SOP for Ocular and Vision Testing at each study visit. Subjects must have each eye refracted prior to performing other vision assessments, for example, prior to visual acuity, contrast vision and HVF testing. Refraction will be performed to obtain the BCVA for each eye.

12.5.2 Visual Acuity

Visual acuity will be assessed at each visit per the study schedule, after refraction. Eligible subjects will include those with eyes able to read letters on the Early Treatment Diabetic Retinopathy Study (ETDRS) chart, defined as on-chart eyes, and eyes unable to read letters on the ETDRS chart, defined as off-chart eyes. Please refer to the SOP for Ocular and Vision Testing for the detailed definition of off- and on-chart eyes.

On-chart eyes are able to read at least 3 letters on the ETDRS chart at 1 meter distance and will thus have an ETDRS score, Snellen BCVA and LogMAR BCVA based on the letters/lines read on the ETDRS chart at 4 and 1 meters' distance.

Off-chart subjects are not able to read at least 3 letters on the ETDRS at 1 meter distance and thus will have an ETDRS score but will not have Snellen visual acuity or LogMAR acuity obtainable based on the number of ETDRS letters/lines read. Off-chart subjects will be tested for count fingers (CF) or hand motion (HM) visual acuity per the SOP for Ocular and Vision Testing.

LogMAR visual acuity will be obtained for off-chart eyes based on Karanjia et al. 2014 (Karanjia 2014). Please see the SOP for Ocular and Vision Testing for full details. Briefly, the finger/hand dimensions of the examiner will be measured. The examiner will present the fingers/hand to assess CF or HM vision and will measure the distance at which the subject accurately counted fingers or detected HM. The finger/hand dimensions of the examiner and the distance at which CF or HM was performed will be entered into the equation utilized by Karanjia et al. 2014, modified for assessment of visual acuity in meters. The output of interest is the LogMAR BCVA.

For the purposes of the study 1 ETDRS line / 5 ETDRS letters is considered equivalent to 0.1 LogMAR and 1 ETDRS letter is equivalent to 0.02 LogMAR.

12.5.3 Pelli-Robson Contrast Sensitivity

Contrast sensitivity will be assessed with the Pelli Robson Low Vision Contrast Sensitivity chart, per the study schedule. Details of the examination procedure will be provided in the SOP for Ocular and Vision Testing.

12.5.4 Humphrey Visual Field 30-2

Standard, automated perimetry will be obtained with the an HVF Analyzer II (Carl Zeiss Meditec Inc.) using the 30-2 SITA Fast strategy, Stimulus III White, per the study schedule. Details of the examination procedure will be provided in the SOP for Ocular and Vision Testing. A central reading center will perform quality control, analysis and interpretation of all HVF.

12.5.5 Goldmann Applanation Tonometry for IOP

IOP will be measured per the study schedule and the Intravitreal GS010/Placebo Injection Guide, by Goldmann applanation tonometry. Topical fluorescein will be utilized per standard local practices for the Goldmann applanation procedure. Details of the examination procedure will be provided in the SOP for Ocular and Vision Testing.

12.5.6 Slit Lamp Examination (Pre- and Post-Pupil Dilation)

Slit lamp biomicroscopy examination will be performed per local standard medical examination techniques, pre- and post-pupil dilation, per the study schedule and the Intravitreal GS010/Placebo Injection Guide. Details of the examination procedure will be provided in the SOP for Ocular and Vision Testing and the visit worksheets will provide a guidance for the examination.

12.5.7 Color Fundus Photo

Color fundus photo of the posterior pole will be obtained at each study visit per the study schedule. Details of the examination procedure will be provided in the SOP for Ocular and Vision Testing. A central reading center will perform quality control, analysis and interpretation of all color fundus photos.

12.5.8 Spectral-Domain Optical Coherence Tomography

SD-OCT will be obtained with the Spectralis® OCT (Heidelberg Engineering). Parameters will be obtained for the optic nerve and RNFL and the posterior pole, per standard protocols included in the Spectralis® software. Details of the examination procedure will be provided in a specific SOP. A central reading center will perform quality control, analysis and interpretation of all SD-OCT data.

12.5.9 Fluorescein Angiography

Fluorescein angiography (FA) may be obtained per the discretion of the Investigator for significant vitreous inflammation or posterior uveitis or as deemed necessary per the investigator for the management of the subject's clinical condition. FA will be performed per the local standard practices. A central reading center will perform quality control, analysis and interpretation of all FA performed.

13. Study Drug Management

Please refer to the following study-specific guides/manuals for full details:

- GS010 and Placebo Reconstitution Protocol
- Pharmacy Manual
- Intravitreal GS010/Placebo Injection Guide

13.1 Description of GS010

AAV is a small parvovirus that infects humans and other primate species. The vector used in this study is a recombinant form of the serotype 2 of the AAV2 vector, rAAV2/2, containing a modified cDNA, encoding the human wild type mitochondrial NADH Dehydrogenase 4 protein, under the control of the cytomegalovirus immediate early promoter in an intron-containing expression cassette (beta globin intron, HBB2), flanked by the virus inverted terminal repeats. The construct includes the cis-acting elements of the human *COX10* mRNA (mitochondrial targeting sequence 5' of the complementary DNA [cDNA], and the 3'untranslated region at the 3' end of the cDNA) ensuring efficient targeting of the mRNA to mitochondria and delivery of the corresponding protein to the mitochondria.

13.2 Description of the Placebo

The placebo is a Balanced sterile Saline Solution (BSS) formulated by the Contract Manufacturing Organization (CMO). The BSS is a sterile, apyrogenic solution used for ocular surgery. It is filled in the same vials as GS010 and frozen at ≤ -70°C ready for intended medical use. Please refer to the Pharmacy Manual for full details.

13.3 Formulation of GS010

GS010 (rAAV2/2-*ND4*) Drug Product is a sterile suspension of concentrated and purified virus vector formulated in Balanced Saline Solution (BSS) plus 0.001% Pluronic F68®. GS010 is stored at ≤ -70°C. It is filled in vials ready for intended medical use. Please refer to the Pharmacy Manual for full details.

13.4 Formulation of the Placebo

Table 2: Formulation of the Placebo Intravitreal Injection

Active Ingredients	Excipients
- Magnesium chloride - Sodium chloride - Calcium chloride - Potassium chloride - Sodium acetate - Sodium citrate	- Water for injection - Sodium hydroxide - Hydrogen chloride

13.5 Storage of GS010 and Placebo

GS010 and Placebo are stored at ≤-70°C in a freezer under the supervision of the study pharmacist or the Investigator. Vials of GS010 and Placebo will only be dispensed with the written authorization of the Investigator or a specifically designated delegate of the Investigator. Please refer to the Pharmacy Manual for full details.

13.6 Packaging and Labeling

GS010 and the Placebo are individually packaged as directed in the IMPD.

The GS010 package contains one vial of GS010.

The placebo package contains one vial of placebo.

The primary label on the vial as well as secondary container labeling is in the country-specific language for each site. The labels are compliant with local regulatory requirements. The primary and secondary packaging together comply with the labeling requirements.

Packaging of IMPs will be compliant with the International Air Transport Association (IATA) regulation for genetically modified organisms.

13.7 Dose and Administration

Please refer to the Intravitreal GS010/Placebo Injection Guide, for full details.

13.7.1 IMP GS010

GS010 will be administered via standard intravitreal injection under local (i.e. topical) anesthesia. Any eye allocated to treatment with GS010 will receive a single IVT injection of the IMP. GS010 will be administered per the study schedule. GS010 will be administered at a dose of [redacted]/eye in a final volume of [redacted].

13.7.2 IMP Placebo

The placebo will be administered via standard intravitreal injection, under local (i.e. topical) anesthesia. Any eye allocated to placebo will receive a single IVT injection of placebo. The placebo

will be administered per the study schedule. The placebo will be administered in a final volume of [redacted].

13.8 Dispensing and Accountability

The IMPs (GS010 and placebo) will only be dispensed, per the Investigator's prescription, to eligible subjects who meet all selection/non-selection and inclusion/exclusion criteria and according to the study schedule and treatment scheme.

The IMPs (GS010 and placebo) will be tracked during the handling process from prescription to destruction (through dispensation, reconstitution, administration and decontamination).

Accountability for GS010 and placebo is the responsibility of the Investigator. This responsibility however may be delegated to the Pharmacist(s). This delegation will be documented in the Site Trial Master file.

The Pharmacist or the person responsible for study medication management will keep an IMPs accountability log detailing the dates, batch number and quantity dispensed for each subject. Accountability records will be verified by the study monitor during site visits.

At the end of the study, all unused vials of the IMPs (GS010 and placebo) will be destroyed at the investigation center following internal procedures for Genetically Modified Organisms (GMO). A copy of the certificate of destruction should be made available to the sponsor.

13.9 Prior and Concomitant Therapies

Prior therapy is defined as prescription and non-prescription medications and preparations, including health and/or dietary supplements taken within 30 days prior to signing the informed consent, but discontinued prior to signing informed consent.

Concomitant therapy is defined as prescription and non-prescription medications and preparations, health and/or dietary supplements other than GS010 that the subject receives during the study (i.e. any time after the signing of informed consent). This includes any medication started prior to signing informed consent and continued after signing the informed consent form.

Idebenone must be discontinued at least seven days prior to Inclusion Visit 2 and is not allowed to be taken during the study period. No other medications are specifically forbidden during the study.

13.9.1 Immune Modulating Regimen

All subjects will receive a peri-treatment systemic oral immune modulating regimen for the prevention or diminution of ocular inflammation related to IVT injection of GS010. The immune modulating regimen will consist of oral corticosteroid starting 2-days prior to the initial treatment administration to any eye of the subject and will be completed after a 28-day immune modulating regimen. The following regimen will be administered:

- Immune modulating agent: corticosteroid
- Route of administration: per os (p.o.; i.e. orally)
- Administration regimen:

- 40 milligrams (mg) daily for 1 week, starting two days prior to treatment administration to any eye of the subject, then
- 30 mg daily for 1 week, then
- 20 mg daily for 1 week, then
- 10 mg daily for one week → then stop

13.10 Method of Assigning Treatment

An IRS will be utilized to enroll and randomize subjects. Each subject will receive a unique subject identification number assigned at Screening Visit (Visit 1), which will serve as the subject identifier throughout the study. The subject number will be required for all communication between the Investigator (or designee) and the IRS. Subject numbers will be tracked via the IRS. Subjects who meet all selection and inclusion criteria will then be randomized via the IRS, based on a pre-defined central randomization scheme. An IRS User Guide will describe all steps for enrollment and randomization of the subjects.

As described in section 9.1, the duration of vision loss of each eye will be determined and documented at the Screening and Inclusion Visits (Visit 1 and Visit 2; for Visit 2 only if applicable in the setting of new onset vision loss in an eye previously unaffected by clinically manifested visual acuity loss Visit 1). The eye with the longest duration of vision loss will be the first affected eye and the eye with the shortest duration of vision loss will be the second affected eye. Eyes that have not yet experienced clinically manifested decline in visual acuity will be considered “not yet affected” eyes and have a value of zero days for vision loss duration. Subjects reporting simultaneous onset of vision loss in both of their eyes will have an equal vision loss duration for both eyes.

Subjects meeting all eligibility criteria for selection and inclusion will be randomized to treatment arm 1 (TARM1) or treatment arm 2 (TARM2) in a 1:1 allocation (see Figure 1). Subjects in TARM1 will receive intravitreal GS010 in their first affected eye and their second affected/not yet affected eye. Subjects in TARM2 will receive GS010 in their first affected eye and placebo intravitreal injection in their second affected/not yet affected eye.

Subjects with non-simultaneous onset of vison loss, for which a first affected eye and a second affected/not yet affected eye are designated based on non-equal vision loss duration, will be randomly allocated to TARM1 or TARM2 via the IRS system.

Subjects with simultaneous onset of vision loss in both eyes (i.e. equal vision loss duration for both eyes) will first be randomly allocated to TARM1 or TARM2 via the IRS system. Subsequently, for these subjects, the right or left eye will be randomly selected to serve as the second affected/not yet affected eye. If the right eye is selected randomly as the second affected/not yet affected eye, the left eye will be designated as the first affected eye, and vice versa.

13.10.1 Determination of the Best- and Worst-Seeing Eye

At Inclusion Visit (Visit 2), the best-seeing and worst-seeing eyes of each subject will be determined, based on the baseline vision testing performed at Visit 2. This will allow for assessment of the presence of a difference of the treatment effect in best- and worst-seeing eyes. A pre-defined algorithm for determining the best- and worst-seeing eyes will be utilized as follows:

1. LogMAR BCVA: On-chart eyes and off-chart eyes will have a LogMAR BCVA per the study's SOP for visual acuity testing. The eye of the subject with the better (i.e. lower or more negative) LogMAR BCVA will be the better-seeing eye of the subject. If both eyes have an equal LogMAR acuity the second criterion will be utilized.
2. SD-OCT parameters (to be used if there is no inter-eye difference based on Criterion 1):
    i. The initial SD-OCT parameter will be the total volume of the RGC layer of the ETDRS macula area. The better-seeing eye will have the greater volume and quadrant thickness, unless there is macula edema. A difference of ≥ 5% is considered significant to determine greater volume.
    ii. The second SD-OCT parameter (to be used if there is no inter-eye difference in criteria 2i) will be the combined volume of the RGC layer of the inner and outer nasal quadrants of the macula. The better-seeing eye will be the eye with greater volume. A difference of ≥ 5% is considered significant to determine greater volume.
3. Log of Contrast Sensitivity (LogCS) obtained with the Pelli Robson Low Vision Contrast Sensitivity Chart (to be used if there is no inter-eye difference in criteria 1 or 2). The eye with the best LogCS score is the better-seeing eye.
4. If the eyes are equal based on criteria 1 through 3, the best-seeing eye will be based on the subject's opinion.

13.11 Masking

Masking of both the subjects and the investigation team for the primary analysis, will be accomplished using a placebo intravitreal injection and the randomized allocation of treatment for second affected/not yet affected eyes. Only the pharmacist and the pharmacy team will be aware of the treatment allocation. All other study personnel, including but not limited to, the Investigator, designees or delegates of the Investigator (except the pharmacist/pharmacy team), the physician and allied health professionals performing the IVT injection of the IMPs (GS010 and placebo), and the ophthalmic technicians/orthoptists/optometrists or other study personnel performing the visual tests and ocular examinations, will be masked to treatment allocation.

To maintain the integrity of the ongoing study, individuals who are directly involved in study conduct and data management (e.g., personnel at investigational sites, Medical Safety Physician, Clinical Operations, and Data Management at CRO and Sponsor level) will be kept masked to the treatment allocation up to Visit 12 (Year 1.5). The Sponsor and CRO will be formally unmasked for the primary efficacy analysis at 1.5 years (Week 78) to allow an early registration if results meet the expectations.

Unmasked individual pharmacovigilance CRO personnel may know the treatment allocation of a given subject. No Sponsor or CRO personnel in charge of the conduct of the study (e.g. CRA, data manager, study manager) will have access to treatment information.

13.12 Procedure for Unmasking

The treatment allocation (intravitreal GS010 or placebo) should not be unmasked (revealed) unless knowledge of the subject's eye treatment is required for the subject's clinical care and safety. The Investigator will be provided with instructions as to how to unmask the treatment allocation via the IRS. In the unlikely event that knowledge of the treatment assignment of an eye(s) is necessary in order to care for a subject, the Investigator may decide to unmask the treatment allocation for safety reasons. If the treatment assignment is unmasked, the Sponsor and the assigned Clinical Research Associate (CRA) must be notified within 24 hours. Documentation of the unmasking of a treatment assignment should be recorded in the subject's medical record with the reason for unmasking, the date and time of the unmasking, and the names of the personnel involved.

14. Adverse Events

Subjects' condition will be monitored throughout the study. Overall incidence of AEs and SAEs will be evaluated for the entire study.

An AE is defined as any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether related to the medicinal (investigational) product, or not. Any event or laboratory abnormality that leads to a medical intervention, including withdrawal of IMP or significant additional concomitant therapy, will be considered an AE. Worsening of visual acuity determined by the Investigator to be due to progression of LHON will not be considered an AE.

AEs should be volunteered by the subject, be observed from examination of the subject at a clinic visit or be from observations of clinically significant laboratory values or other/special examination abnormal values. AEs will not be solicited by the use of a specific list of anticipated events.

All AEs are to be assessed and recorded in a timely manner. Each AE is to be documented regarding severity, date of occurrence, duration, treatment, and outcome. Furthermore, each AE is to be classified as being serious or non-serious. In addition, the Investigator must assess whether the AE is drug-related or not or is related to study procedures, or not. Changes in severity of AEs and resolution dates should be documented as separate events.

AEs will be captured from the first study-related procedure (ICF signature) through to the completion of the protocol-defined safety follow-up as defined in sections 14.1 and 15.3.

Surgical procedures planned before enrolment of the subject in the study, are not considered AEs if the condition was known before study inclusion. In this case, the medical condition should be reported in the subject's medical history at Screening Visit 1.

Intermittent AEs will not be reported as multiple AEs. The definition of an intermittent AE is "a recurring event with the same severity, frequency, and causality."

Clinically relevant abnormal findings observed during vital signs measurements or physical examinations that are not related to the subject's documented medical history, will be reported as AEs.

14.1 Reporting and Documentation of Adverse Events

14.1.1 Reporting

At each visit, any AE directly observed or mentioned by the subject will be reported by the Investigator or designee on the page "Adverse Events" of the eCRF (also called the "AE page"). The following items must be documented:

Nature of the event with self-explanatory and concise medical terminology (indicate a diagnosis or syndrome instead of symptoms). A single diagnostic term per AE should be utilized; each diagnostic verbatim term will be counted as a single AE.

Date of onset and date of resolution (i.e. actual dates when the event starts and is resolved rather than dates when the Investigator is informed).

- Severity.
- Relationship to IMP.
- Relationship to study procedure.
- Outcome.
- Seriousness.
- Action taken regarding the IMP.
- Any action taken regarding the event, whether by the Investigator, the subject (as reported by the subject), or by another physician (as reported by the subject or in the subject's medical record).

AEs requiring therapy must be treated with recognized standards of medical care to protect the health and well-being of the subject. Any treatment given will be reported on the page "Concomitant medication" of the eCRF.

14.1.1.1 Assessment of Severity

The severity of clinical AE is graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The CTCAE scale will be provided to Investigators.

Should an event be missing in one of the scales, the following 3-point scale must be used:

- Mild: Discomfort noticed, but no disruption of normal daily activity.
- Moderate: Discomfort sufficient to affect normal daily activity.
- Severe: Inability to work or perform normal daily activity.

Table 3 shows the correspondence between the severity levels of the CTCAE grades and the 3-Point Scale grades.

Table 3: Correspondence Between CTCAE and 3-Point Scale Severity Levels

CTCAE	**3-Point Scale**
1	Mild
2	Moderate
3/4/5	Severe

14.1.1.2 Relationship to the IMP and/or Study Procedures

The relationship of each AE to the IMP and/or study procedures will be evaluated as follows:

- Unrelated: There is evidence of relationship to a cause other than the IMP/study procedures. Does not meet criteria listed under unlikely, possible or probable.
- Unlikely: Does not follow a reasonable temporal sequence from administration. Is most likely produced by the subject's clinical state or by environmental factors or other therapies administered.
- Possible: Follows a reasonable temporal sequence from administration. Is not likely produced by the subject's clinical state or by environmental factors or other therapies administered.
- Probable: Follows a reasonable temporal sequence from administration. Clear-cut temporal association with IMP.

14.1.1.3 Outcome

The outcome of each AE will be rated as follows:

- Recovered.
- Not recovered.
- Recovered with sequelae.
- Fatal. This outcome is to be used only for the event leading to death. The outcome of all other events at the time of the death must be reported. The outcome of ongoing ones is reported as "not recovered."
- Unknown.
- Worsening. This outcome is used when an AE worsens. The new status of the event is documented as another AE on the AE page in the eCRF.

14.1.2 Documentation

AEs must be reported in the source document with at least the nature of the event, the diagnosis, start date, stop date and the treatment (if applicable) of the event.

14.2 Ocular Inflammation

As noted, ocular inflammation is an expected adverse side effect of intravitreal administration of GS010 and occurs in most subjects in which GS010 is administered to the intravitreal compartment. Ocular inflammation may encompass anterior uveitis (anterior chamber inflammation), intermediate uveitis (vitreous inflammation, vitritis) or less likely, posterior uveitis. It is therefore of clinical pertinence to specify the accurate and standard documentation of the clinical aspects of ocular inflammation.

Specific, standardized schemes for assessing anatomic location, severity, and clinical evolution of ocular inflammation will be employed. The Standardization of Uveitis Nomenclature (SUN) Working Group provided standardized methods for anatomic classification of uveitis, a grading scheme for uveitis and terminology describing the activity of the uveitis (Jabs 2005, Table 4, Table 5, Table 6, and Table 7 below). These are provided below and must be used by the clinical investigation team to assess and document the presence and evolution of ocular inflammation.

Table 4: Anatomic Classification of Uveitis (SUN Working Group, Jabs 2015)

Type	Primary Site of Inflammation[a]	Includes
Anterior uveitis	Anterior chamber	Iritis Iridocyclitis Anterior cyclitis
Intermediate uveitis	Vitreous	Pars planitis Posterior cyclitis Hyalitis
Posterior uveitis	Retina or choroid	Focal, multifocal, or diffuse choroiditis Chorioretinitis Retinochoroiditis Retinitis Neuroretinitis
Panuveitis	Anterior chamber, vitreous, and retina or choroid	

SUN = Standardization of Uveitis Nomenclature
[a] As determined clinically. Adapted from the International Uveitis Study group anatomic classification.

Table 5: Grading Scheme for Anterior Chamber Cells (SUN Working Group, Jabs 2015)

Grade	Cells in Field[a]
0	<1
0.5+	1-5
1+	6-15
2+	16-25
3+	26-50
4+	>50

SUN = Standardization of Uveitis Nomenclature
[a] Field size is a 1 mm by 1 mm slit beam.

Grading of vitreous cells in the anterior vitreous will be performed based on the slit lamp examination utilizing a 1 millimeter by 1 millimeter slit lamp beam and the same grading scheme utilized for anterior chamber cells (see Table 5).

Table 6: Grading Scheme for Anterior Chamber Flare (SUN Working Group, Jabs 2015)

Grade	Description
0	None
1+	Faint
2+	Moderate (iris and lens details clear)
3+	Marked (iris and lens details hazy)
4+	Intense (fibrin or plastic aqueous)

SUN = Standardization of Uveitis Nomenclature

Table 7: Activity of Uveitis Terminology (SUN Working Group, Jabs 2015)

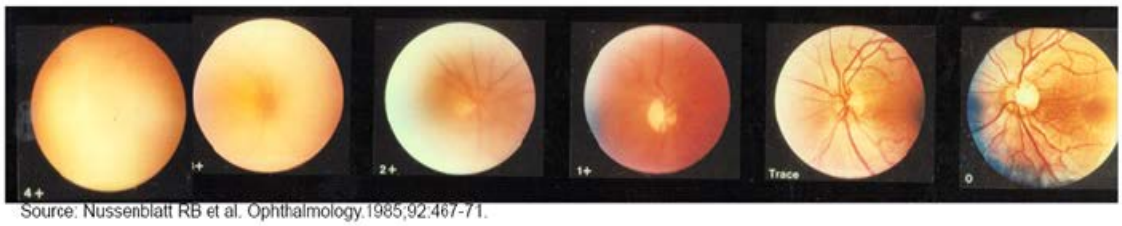
Term	Definition
Inactive	Grade 0 cells[a]
Worsening Activity	Two-step increase in level of inflammation (e.g. anterior chamber cells, vitreous haze) or increase from Grade 3+ to Grade 4+.
Improved Activity	Two-step decrease in level of inflammation (e.g. anterior chamber cells, vitreous haze) or decrease to Grade 0.
Remission	Inactive disease for ≥ 3months after discontinuing treatments for eye disease

SUN = Standardization of Uveitis Nomenclature
[a] Applies to anterior chamber inflammation.

Grading of vitreous haze will be performed in a standardized fashion by the central ophthalmology reading center, based on color fundus photos of the posterior pole. Baseline color fundus photos will be obtained at all visits for all subjects. The National Institutes of Health grading system (Nussenblatt 1985, Table 8) will be used to grade the vitreous haze on the fundus photo. and photos will serve as the basis for vitreous haze grading.

Table 8: National Institutes of Health Grading System for Vitreous Haze

Grade	Amount of Vitreous Flare/Haze
0	No flare
0.5+	Trace
1+	Clear optic disc and vessels, hazy nerve fiber layer
2+	Hazy optic disk and vessels
3+	Optic disc visible
4+	Optic disc not visible
Quality Unsatisfactory	Photo quality inadequate for vitreous inflammation grading
Not Performed	Photograph not performed



Source: Nussenblatt RB et al. Ophthalmology.1985;92:467-71.

14.3 Laboratory Changes

A laboratory abnormality is reported as an AE if it is out of range, considered by the Investigator as clinically significant (i.e. with clinical manifestations or requiring treatment or clinical management) and confirmed by a repeat measurement (if relevant). Worsening of laboratory parameters from pre-drug administration state will be considered on the same basis.

14.4 Concomitant Medications

The Investigator or designee will record any concomitant therapies given for the treatment of AEs on the concomitant medication page of the subject's source document and eCRF. During the study, any medication taken by the subject is to be reported by the subject and noted on the subject's source document and eCRF. Any changes in concomitant therapy during the study will be documented, including cessation of therapy, initiation of therapy, and dose changes.

14.5 Adverse Event Follow-Up

AEs occurring during the study must be followed until resolution or the last visit planned by the protocol. AEs not resolved by the last visit planned by the protocol will be listed in the eCRF as continuing/not resolved.

Subjects who experience the onset of an AE thought to be related to IMP after the final study visit will be followed up until the resolution of the AE or for 30 days after the AE occurs, whichever

comes first. AEs not resolved after 30 days will be listed in the eCRF as continuing/not resolved. SAEs thought to be related to IMP occurring after the final study visit must be reported as described in Section 15.3.

14.6 Pregnancy

If a female subject or the partner of a male subject believes she is pregnant (e.g. missed period, self-administered pregnancy test) the subject will be instructed to present for medical follow-up within 48 hours to undergo a serum pregnancy test. All confirmed pregnancies that occur within this study will be followed until resolution (i.e. termination [voluntary or spontaneous] or birth).

If the pregnancy is confirmed before the subject received IMP, the subject will be excluded from study participation and will not receive IMP.

Pregnancy (without associated unexpected or adverse sequelae) is not a reportable AE but must be reported to the Sponsor within 24 hours of the Investigator or study staff first becoming aware of the subject's condition.

15. Serious Adverse Events

15.1 Definition of Serious Adverse Events

An SAE is any AE that meets any of the following criteria:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect in the offspring of a subject who received the IMP

Other important medical events that may not result in death, be life-threatening, or require hospitalization, may be considered an SAE 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. Examples of such events include:

- Intensive treatment in an emergency room or at home for allergic bronchospasm
- Blood dyscrasias
- Convulsions that do not result in inpatient hospitalization

15.2 Definition of Terms

Life threatening: an AE is life threatening if the subject was at immediate risk of death from the event as it occurred (i.e. it does not include a reaction that might have caused death if it had occurred in a more serious form). For example, drug induced hepatitis that resolved without evidence of hepatic failure would not be considered life threatening, even though drug induced hepatitis can be fatal.

Hospitalization: AEs requiring hospitalization should be considered SAEs. Hospitalization for elective surgery for a pre-existing condition that has not worsened, or hospitalization for routine clinical procedures that are not the result of an AE need not be considered AEs or SAEs. Any untoward event occurring during the procedure must be reported as an AE, either 'serious' or 'non-serious' according to the usual criteria.

In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. When in doubt as to whether 'hospitalization' occurred, or was necessary, the AE should be considered serious.

Disability/incapacitating: an AE is incapacitating or disabling if the experience results in a substantial and/or permanent disruption of the subject's ability to carry out normal life functions.

15.3 Reporting of Serious Adverse Events

Any SAE occurring during the study, i.e. between the ICF signature and the end of study visit, **MUST be reported to the Sponsor** or their representative. The Investigator must complete and fax a "Serious Adverse Event Form" to the Sponsor or their designee within **24 HOURS** of occurrence or knowledge of the event.

An Investigator's delegate may complete the SAE form; however, the Principal Investigator or delegate physician must sign it. The form can be sent to the Sponsor or their designee with the delegate's signature if the Principal Investigator/delegate physician's signature cannot be obtained within one working day. The Principal Investigator/delegate physician's signature must be obtained as soon as possible, as must his/her evaluation of the relationship to the IMP. The signed form must be faxed and/or emailed to the Sponsor or their designee immediately.

The SAE form must be completed in English.

15.3.1 Follow-Up

If follow-up information is not available at the time of the event, this information must be forwarded to the Sponsor or their designee within one day of knowledge. The Sponsor or their designee may request information as needed.

15.3.2 Post-Study Serious Adverse Events

Any SAE occurring after the end of study visit and that is considered by the Investigator to be possibly or probably related to the IMP must be reported to the Sponsor or their designee as described above.

15.3.3 Notification to Regulatory Authorities / IRB / EC / Gene Therapy Regulatory Bodies

The Sponsor or their designee is responsible for notifying serious and unexpected AEs to Health Authorities and to IRBs/IECs in accordance with local law.

Gene Therapy Bodies will be informed as required locally.

15.3.4 Information for Investigator

When an SAE has been reported to Competent Authorities, the Sponsor or their designee will inform all other Investigators working in this study, as well as those working with GS010 in other studies, in accordance with local laws and regulations.

15.3.5 Documentation

All SAEs will be reported on the AE pages of the CRF using the same information as documented on the SAE form and source documents. Copies of SAE forms will be filed in the Investigator Site File, along with copies of any correspondence with the IRB/IEC. The Investigator Site File will also include copies of notification letters and/or faxes/emails of forms sent to Competent Authorities and Gene Therapy Bodies if appropriate.

16. Statistics

16.1 General Considerations

A statistical analysis plan (SAP) will be made final after the protocol is approved and before database lock occurs. The SAP will provide further details regarding the endpoints, data handling rules, and the statistical methods to be used to address all study objectives. The SAP will also include formats for the summary and analysis tables, listings, and graphical displays.

Data for quantitative variables will be summarized using descriptive statistics (number of subjects, arithmetic mean, standard deviation (SD), median, first quartile (Q1), third quartile (Q3), minimum and maximum). Data for binary variables (e.g. responder analysis) will be presented by using counts and percentages. Least square (LS) means, 95% confidence interval, and P-values will be derived from models including covariates (see primary model) in addition to treatment.

16.2 Sample Size

Sample size calculation is based on the primary endpoint which is the change from baseline to 1.5 years post-treatment of the visual acuity (LogMAR). Masked preliminary data of the Phase III RESCUE and REVERSE sham-controlled studies of GS010 and monitored data from the Phase I/IIa safety and tolerability study of GS010 are used to estimate the standard deviation of the change from baseline to each available visit. These data do not take into account a potential treatment effect in the RESCUE and REVERSE studies and if there is an effect the standard deviation may be over-estimated.

From this masked preliminary analysis, a SD of 0.55 LogMAR has been estimated for the LogMAR change from baseline; this is reduced to 0.50 with the inclusion of baseline LogMAR as a covariate. Using this reduced value for SD and a treatment effect of 0.3 LogMAR in the change from baseline, the requested sample size to reach a power of 80% (alpha = 0.05 two-sided) according to an ANCOVA model including the baseline LogMAR value as covariate has been estimated, using SAS PROC POWER, to be 45 evaluable subjects per treatment group (i.e. 90 subjects in total).

16.3 Populations for Analysis

16.3.1 All subjects enrolled set

The all subjects enrolled set will contain all subjects who provide informed consent for this study.

16.3.2 Intent to Treat Population

The intention-to-treat (ITT) analysis population will consist of all randomized subjects. The analyses will be based on the planned treatment (as randomized). This ITT population, which corresponds to the definition of International Conference on Harmonization (ICH) Statistical Principles for Clinical Trials E9, is the primary efficacy analysis population. All missing endpoint data using the ITT population will be imputed.

16.3.3 Modified Intent to Treat Population

The modified intent-to-treat population (mITT) will consist of all subjects who are randomized and received study treatment (GS010 or placebo) in the second affected /not yet affected eye. In the case of an error in treatment allocation, the randomized treatment will be used in the analyses. Missing data will be imputed. The mITT population is supportive of the ITT analysis population. If all randomized subjects are treated, then this population will not be employed.

16.3.4 Per Protocol Population

The Per Protocol (PP) population will consist of all subjects who are randomized and received study treatment (GS010 or placebo) in the second affected /not yet affected eye. In the case of an error in treatment allocation, i.e. GS010 is administered to the eye allocated to receive placebo (or vice versa), the actual treatment received will be used in the analyses. Subjects identified with major protocol deviations, which are judged to potentially interfere with the interpretation of the treatment effect, will be removed from the PP analysis population. Removal of subjects from the PP population will be performed prior to treatment unmasking. Missing data will not be imputed. The PP population is supportive of the ITT analysis population.

16.3.5 Safety Analysis Population

The safety population is defined as those subjects who received GS010 or placebo in at least one eye. This population will be used as the population for all safety analyses.

16.3.6 Follow-up Population

The follow-up population is defined as those subjects who completed Visit 13 and enrolled in the Post-Treatment Follow-up Period 2. This population will be used to examine both safety and efficacy for up to 5 years post treatment.

16.4 Statistical Methods

16.4.1 Primary Efficacy Analysis

The primary analysis will be based on the following:

16.4.1.1 Primary Population

The primary set of subjects will be the ITT population.

16.4.1.2 Primary Eye

The primary eye will be the second affected/not yet affected eye. For subjects reporting simultaneous onset of vision loss in both of their eyes, indicating that the subject was unable to distinguish which is the first affected eye and which is the second affected/not yet affected eye, the right or left eye will be selected randomly to serve as the second affected/not yet affected eye. If the right eye is selected randomly as the second affected/not yet affected eye, the left eye will be designated as the first affected eye, and vice versa (see Section 13.10). If the second affected eye

did not receive the correct treatment (as set in the randomization scheme) then the subject will be assigned to the arm corresponding to the treatment actually received. Consequently, if subject received GS010 in both eyes then the subject is assigned to the arm with both eyes treated with GS010. If one eye received a placebo injection, then the subject is assigned to the treatment arm which receives placebo. In this case, the primary eye will be the eye receiving the placebo even if it is the first affected eye instead of the second affected/not yet affected eye. In other words, in case of assignment error, the treatment prevails over the order of affection of eye (*i.e.* first or second affected/not yet affected eye corresponding to secondary or primary eye, respectively).

16.4.1.3 Primary Assessment Criterion

The primary assessment criterion is the BCVA reported with LogMAR. The on-chart visual acuity (ETDRS) and off- chart visual acuity will be transformed into LogMAR according to the rules presented in the SAP.

16.4.1.4 Primary Time Point

The primary time point is 1.5-Year post-treatment. The other time points are mainly "operational" time points, particularly useful for assessing the time course of the response and time to event analyses.

16.4.1.5 Primary Individual Response

The primary individual response of interest is the change from baseline to 1.5-Year post-treatment. A negative change from baseline in LogMAR is an improvement and a positive change from baseline is a worsening.

16.4.1.6 Primary Measure of the Treatment Effect

The primary measure of treatment effect is the between treatment (GS010 and placebo) difference in the mean of the primary response, assessed with an ANCOVA model.

16.4.1.7 Primary Covariates

The primary model will use the LogMAR value at baseline of the second affected/not yet affected eye (i.e. the primary eye) as covariate.

16.4.1.8 Primary Model and Test

The primary analysis is analysis of covariance. The response is the change from baseline at 1.5-years post-treatment. The test will compare the between treatment difference in means adjusted for the LogMAR baseline value and duration of disease, and including the treatment group as a fixed effect.

16.4.1.9 Threshold of Clinical Pertinence

The average of the adjusted difference in the mean change from baseline to 1.5 years post treatment between the treatments will be reported with the standard errors of the mean, two-sided 95% confidence interval, and P-value obtained from the multiple imputed datasets and analysis described in the SAP. An average adjusted difference of 0.30 LogMAR in favor of GS010 is considered to be a clinically significant advantage.

16.4.1.10 Primary Significance Level

The analysis will be performed using a significance level of 0.05 (two-sided).

16.4.1.11 Supportive Analyses

The list of supportive analyses will be presented in the SAP. The list will include the same analysis performed on the per protocol set of subjects and on the modified intent-to-treat (mITT) population. Possibly, a rank ANCOVA on the ITT set of subjects will be conducted in case of major outliers. Major outliers are defined as observations associated with an absolute studentized residual above 5. The last supportive analysis is the primary analysis with the following covariates added to the model: the duration of vision loss at baseline of the second affected/not yet affected eye, the baseline GCL volume, the region, the region-by-treatment interaction.

16.4.1.12 Sensitivity Analysis

For ITT and mITT analyses, missing data will be imputed using multiple imputations.

16.4.1.13 Handling of Centers

Sites with fewer than 5 subjects will be pooled per region created during the masked review of data to obtain pooled centers of approximately the same number of subjects.

16.4.1.14 Handling of Missing Data

In case of missing data up to and including 1.5 years in the primary (second affected/not yet affected) eye, multiple imputation will be used as the primary method of imputation for the following endpoints: BCVA measured in LogMAR, Pelli-Robson CS, and HVF 30-2.

Multiple imputation using ten imputations of missing endpoint value will be produced using SAS PROC MI followed by PROC MIANALYZE for both missing at random and missing not at random missing data mechanisms. The seed used to begin random number generation will be 20190219. The Markov chain Monte Carlo method will be used to compute 10 full imputation data sets using the following baseline predictor variables: baseline of endpoint being imputed, duration vision loss in eye, age and gender. Baseline LogMAR and baseline contrast sensitivity will be included in all models.

Last observation carried forward method (LOCF) will be used as secondary method of imputing missing data analysis.

In summaries of data by visits for ITT or mITT populations, the average of the multiply imputed values from PROC MI, rounded to the level of the non-missing data, will be used.

16.4.1.15 Interim Analyses

The DSMB will periodically review safety and may recommend the discontinuation of the study for safety reasons.

Although there is not a planned interim analysis of efficacy the study will be formally unmasked for the primary efficacy analysis at 1.5 years (Week 78) following Sponsor Authorization of this SAP and interim Database Lock to allow early registration if results meet the expectations.

16.4.1.16 Final Analysis

All final, planned analyses identified in this SAP will be performed by IQVIA Biostatistics following the final Database Lock. All efficacy analyses performed at 2, 3, 4 and 5 years post dose are considered exploratory and will be supportive of the primary efficacy analysis.

16.4.1.17 Timing of the Primary Analysis

Primary endpoint analysis will be performed as soon as the last subject completes the 1.5 years LogMAR assessment and data are cleaned. At that point, all subjects will have been recruited and followed for at least 1.5-years except in case of premature withdrawal. The study will be formally unmasked for the efficacy analysis at 1.5 years (Week 78) to allow an early registration if results meet the expectations. The study will continue up to the scheduled end (5 Years).

16.4.2 Secondary Efficacy Analyses

The secondary efficacy endpoints are the following:

1. Change from baseline in LogMAR BCVA at each timepoint of the follow-up period up to 5 years post-treatment.
2. Response status at each timepoint of the follow-up period up to 5 years post-treatment. Definitions of responder eyes include:
    a. Eyes whose LogMAR BCVA improves (i.e. decreases) by ≥ 0.3 LogMAR (equivalent to a gain of ≥ 15 ETDRS letters) compared to baseline.
    b. Eyes whose LogMAR BCVA does not increase (i.e. worsen) by ≥ 0.3 LogMAR (equivalent to eye that lose ≤ 15 ETDRS letters) compared to baseline.
    c. Eyes whose LogMAR visual acuity is < 1.0 (i.e. better than LogMAR 1.0, equivalent to better than Snellen acuity of 20/200).
3. Change from baseline in parameters measured with SD-OCT (including the GCL volume/thickness and the Temporal Quadrant RNFL thickness), HVF 30-2, and Pelli Robson Low Vision Contrast Sensitivity at each timepoint of the follow-up period up to 5 years post-treatment.
4. Change from baseline in Visual Functioning Questionnaire-25 at each post-treatment visit.
5. Change from baseline in 36-Item Short Form Health Survey, version 2 Questionnaire at each post-treatment visit.

All secondary endpoints will be analyzed for the second affected/not yet affected eyes (primary eye), but exploratory efficacy analyses will also be conducted for the first affected eyes.

16.4.2.1 Responder analysis

Response status will be assessed at eye level and subject level.

At eye level, the eye responder status will be assessed for all eyes, and for the following subgroups: first affected eyes GS010; second affected eyes GS010, all eyes GS010 and all eyes Placebo.

At subject level, for subject response, the best response observed in either eye will be considered. Subject response status will be assessed for all subjects, bilaterally GS010 treated subjects and unilaterally GS010 treated subjects.

For each of the response definitions, a logistic model, including factor for treatment group baseline covariate adjustment, will be performed to compare differences in responder rates. An odd ratio less than 0.8 between treatment groups will be considered as a clear clinically meaningful.

LogMAR:
Three definitions of responders for LogMAR are given in Section 8.2 where the first definition is the most important. The first responder definition (Gainer eyes) is an eye whose LogMAR BCVA improves (i.e. decreases) by $\geq$ 0.3 LogMAR:

LogMAR at 1-5 years – LogMAR at Baseline $\leq$ -0.30 = responder eye.

A logistic regression, with adjustment for baseline, will be performed to compare differences in responder rates. An odd ratio less than 0.8 between treatment groups will be considered as a clear clinically pertinent effect. An interaction with region will be assessed. The second definition of responder ("Stabilized eyes") is an eye whose LogMAR BCVA does not worsen (i.e. does not increase) by $\geq +0.3$ LogMAR:

LogMAR at 1.5 Years – LogMAR at Baseline $\leq$ +0.30 = responder eye.

The third definition of interest is exploratory given the precipitous vision loss experienced by the majority of *ND4* LHON subjects. The third definition of responder eye is an eye whose LogMAR visual acuity is better (i.e. lower) than 1.0 (i.e. equivalent to being better than a Snellen acuity of 20/200). Any significant difference in the percentage of second affected/not yet affected responder eyes, receiving GS010 compared to placebo, is pertinent considering the seriousness of the visual impairment. The same analysis as for the first responder definition will be performed for the second and third definitions of responder.

16.4.2.2 Parameters measured with SD-OCT, HVF and contrast sensitivity

Parameters of SD-OCT (e.g. Total and Temporal Quadrant RNFL thickness, GCL volume and thickness and other parameters measured by SD-OCT), parameters of standardized, automated visual fields obtained with HVF Analyzer II (HVF 30-2) (e.g. mean deviation, foveal threshold sensitivity, pattern standard deviation and others), and contrast sensitivity measured with the Pelli-Robson Low Vision Contrast Sensitivity, will be used as secondary endpoints.

These parameters will be analyzed separately using a MMRM model. The MMRM model will include the appropriate baseline measurement as a covariate; stratification factor, treatment group, time point, and treatment group-by-time point interaction as fixed effects; and subjects as random effect. A first-order autoregressive (AR(1)) covariance structure will be used to model the within-patient error and the Kenward-Roger approximation will be used to estimate the degrees of freedom. If this analysis fails to converge, the following covariance structure will be tested one after the other in that order until one converge (remaining covariance structures will not be tested): ARH(1), CS, CSH, TOEP, and TOEPH.

Adjusted difference between the treatment groups will be estimated at each post-treatment time point, along with the two-sided 95% CI and p-value.

16.4.2.3 Quality of Life assessments

Visual Functioning Questionnaire-25 (VFQ-25)

VFQ25 measures will be assessed at the subject level, looking at change from baseline for all subjects and comparing the change from baseline between the two arms (bilaterally treated subjects vs. unilaterally treated subjects).

Composite score and individual subscale scores from VFQ-25 will be summarized using descriptive statistics. Change from baseline at each visit for the composite score and individual subscales scores will also be summarized similarly. Two-sided 95% CI will also be provided. Analysis of covariance, with adjustment for baseline, will be used to analyze change in VFQ25.

36-Item Short Form Health Survey, Version 2 (SF-36v2)

SF-36 measures will be assessed at the subject level, looking at change from baseline for all subjects and comparing the change from baseline between the two arms (bilaterally treated subjects vs. unilaterally treated subjects).

Composite scores and individual domains from SF-36v2 will be summarized using descriptive statistics. Change from baseline at each visit for the composite scores and individual domains will be summarized similarly. Two -sided 95% CI will also be provided. Analysis of covariance, with adjustment for baseline, will be used to analyze change in VFQ25.

16.4.2.4 Exploratory LogMAR BCVA analysis

16.4.2.4.1 Time to Event Analysis

The two events of interest for a time-to-event analysis are the following:

- A sustained decrease of 0.3 LogMAR or more (maintenance of visual acuity)
- A sustained visual acuity of better than 1.0 LogMAR.
- An increased visual acuity is sustained if the threshold is maintained for two successive visits (except for the last visit at which the sustainability is not required). The time to event will be computed based on the corroborating visit.

The time from baseline to the event of interest will be analyzed using a Cox model using baseline LogMAR as a covariate and including the treatment groups as factors. Subjects who do not meet the definition of the event of interest will be censored at the EOS Visit (or early discontinuation visit is subject discontinued early from study). Kaplan Meier tables and graphs will also be used for descriptive purposes Time-to-Event analysis

The time from baseline to the event of interest will be analyzed using a Cox model using baseline LogMAR as a covariate and including the treatment groups as factors. Subjects who do not meet the definition of the event of interest will be censored at the EOS Visit (or early discontinuation visit is subject discontinued early from study). Kaplan Meier tables and graphs will also be used for descriptive purposes

16.4.2.4.2 Time Course of the Response (LogMAR BCVA)

A MMRM model will be used to examine the change in LogMAR BCVA trends over time. The MMRM model will include the appropriate baseline measurement and duration of disease as covariates; treatment group, study day, and treatment group-by-time point interaction as fixed effects; and subjects as random effect. The AR(1) covariance structure will be used to model the within-patient error and the Kenward-Roger approximation will be used to estimate the degrees of freedom. If this analysis fails to converge, the following covariance structure will be tested one after the other in that order until one converge (remaining covariance structures will not be tested): ARH(1), CS, CSH, TOEP, and TOEPH.

To describe time trends, linear, quadratic, and higher order terms and interactions with treatment will be added to the model, given significance or lower-order polynomials.

16.4.2.4.3 Coverage

ANCOVA with the AUC of the change from baseline to 1.5 years in the LogMAR BCVA as response variable, including treatment group and stratification variable as fixed effects and using baseline LogMAR as a covariate, will be performed.

16.4.2.5 Analysis of Other Efficacy Variables

The SAP will include definitions and description analyses for the following variables:

Nadir for LogMAR BCVA and for LogCS

Clinically Relevant Recovery (CRR)

16.4.2.6 Exploratory Efficacy

Primary and secondary analyses will be repeated for the first affected eyes as exploratory efficacy analyses. Prior to unmasking, additional analyses may be defined.

16.4.2.7 Follow-up Population Analyses

Primary and secondary endpoints will be summarized for the second affected eyes for the subset over time, showing change from baseline. Hypothesis testing is not planned.

16.4.3 Safety Analyses

Incidence and severity of all treatment and non-treatment emergent local and systemic AEs and SAEs will be presented by system organ class and preferred term. Summary descriptive statistics will be presented for all continuous variables.

All AEs will be coded using the most up-to-date version of the Medical Dictionary for Regulatory Activities (MedDRA).

The incidence of AEs (number and percent of subjects reporting the AE at least once during the study) will be summarized by body system and preferred term for all AEs, by the Investigator's attribution of relationship to study drug and by severity. The details will be included in the statistical analysis plan.

16.4.3.1 Laboratory Data

At each visit where clinical laboratory assessments are conducted, summary statistics for the absolute laboratory value and the changes from baseline will be presented. Statistical testing will be performed at the end of follow-up/EOS period (i.e. Visit 13 or Visit 16). Shift tables will be presented to compare the shifts between the baseline and post-baseline visits. Clinically significant abnormal lab values for different parameters will be summarized.

16.4.3.2 Vital Signs

Summary statistics for the absolute values and changes from baseline of systolic and diastolic BP, temperature and pulse rate at each visit, will be summarized.

16.4.3.3 Other Variables Related to Safety

Results from 12-lead ECGs will not be analyzed statistically. The results will be determined to be normal or abnormal and by the Investigator for screening and for comparison to post-treatment assessments.

Physical examination results from the screening visit will be categorized as normal or abnormal and shifts from baseline to the most abnormal post-baseline assessment will be summarized by body system.

Concomitant medication verbatim terms on eCRFs will be mapped to Anatomical Therapeutic Chemical Level (ATC) 4 categories and Drug Reference Names using the World Health Organization (WHO) dictionary (version 01SEP2017EB3 or later). Incidence (number and percent of subjects reporting the medication at least once during the study) will be summarized for all concomitant medications.

16.4.3.4 Follow-up Population Safety Analyses

Safety endpoints will be summarized for the subset over time, showing change from baseline, for continuous endpoints. Adverse events will be summarized overall and by study period, permitting an assessment of change over the 5 years of follow-up.

17. Ethics and Responsibilities

17.1 Good Clinical Practice

The current study will comply with the International Conference on Harmonization (ICH), Good Clinical Practice (GCP), and applicable regulatory requirements.

The Investigator is responsible for ensuring that the clinical study is performed in accordance with the protocol, current ICH GCP guidelines, and applicable regulatory and country-specific requirements. GCP is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides the public assurance that the rights, safety, and well-being

of study subjects are protected, consistent with the principles that originated in the Declaration of Helsinki and that the clinical study data are credible.

17.2 Data and Safety Monitoring Board

An independent DSMB will be constituted and responsible for periodically reviewing study data to assure the continued safe conduct of the study. The DSMB will meet to review the data at least every 6 months. Operational and logistical details will be provided in a separate DSMB charter.

17.3 Institutional Review Board/Independent Ethics Committee

The local IRB/IEC to whom an Investigator is responsible has primary responsibility over any clinical trial performed at that location. The protocol will be reviewed by an independent and appropriately constituted IRB/IEC. Study enrolment and protocol-related procedures, which do not form part of the subject's normal clinical treatment, will not be performed until the IRB/IEC of record has provided written approval of the protocol or a modification thereof. The IRB/IEC must be constituted and operate in accordance with the principles and requirements of ICH GCP.

Study drug can only be supplied to the Investigator after documentation of all ethical and legal requirements for starting the study have been received by the Sponsor. This documentation must also include an IRB/IEC membership list that contains members' occupations. If the IRB/IEC will not disclose the names of the committee members, the IRB/IEC Federal-wide Assurance Number may be accepted as a substitute for this list. Formal approval by the IRB/IEC should mention the study title, study code, study site (or region or area of jurisdiction, as applicable), and any other documents reviewed. It must mention the date on which the decision was made and must be officially signed by a committee member.

17.4 Informed Consent

The Investigator will explain the benefits and risks of participation in the study to each subject or the subject's legally acceptable representative and obtain written informed consent. prior to the subject entering the study and before initiation of any study-related procedure. The informed consent document must be read, understood, and signed by the subject. However, if the subject is unable to read the informed consent document, the presence of an Impartial Witness is required to confirm that the contents of the document were explained to the subject. ICH E6 GCP defines an Impartial Witness as "a person, who is independent of the trial, who cannot be unfairly influenced by people involved with the trial, who attends the informed consent process if the subject or the subject's legally acceptable representative cannot read, and who reads the informed consent document and any other written information supplied to the subject." The Impartial Witness must sign the informed consent document in order to attest to the fulfillment of the regulatory requirements as stated in 21 CRF 50.20 – General requirements for informed consent. The subject must always be asked to sign or mark the informed consent signature page regardless of their vision ability.

For studies collecting long-term follow-up observations, the informed consent document must additionally describe the expected duration of the subject's participation, the time intervals between site visits, the procedure to be followed, and the location of the study visits or whether the subjects will be contacted by other means, and the details as to what those contacts will involve (21 CRF 50.25(a)(1)).

If the subject is under the legal age of consent, the potential subject's parent or legal guardian will be given an IRB/IEC-approved informed consent document for review, and the potential subject will be given an IRB/IEC-approved pediatric assent form. Both the potential subject and their parent/legal guardian will be given the opportunity to ask questions. Both the potential subject and their parent/legal guardian will be informed of the subject's right to withdraw from the study at any time without prejudice. The potential subject and their parent/legal guardian should be able to answer simple questions about the study after the ICF and assent form have been reviewed and explained. After this clarification and before any study-specific procedures can be performed, the parent/legal guardian must voluntarily sign and date the ICF to indicate their informed consent for the subject to participate in the study. The subject must sign and date the pediatric assent form to indicate their will to participate in the study. The Investigator must also sign and date both the ICF and the assent form. Prior to participation in the study, the subject's parent/legal guardian will receive copies of the signed and dated consent and assent forms, along with an emergency card with contact information for the Investigator and clinical facility staff in the event of a medical emergency during the trial.

The Sponsor or designee will provide a sample informed consent form. The final, version dated, form must be agreed to by the Sponsor and the IRB/IEC and will contain all elements in the sample form, in language readily understood by the subject. Each subject's original consent form personally signed and dated by the subject or by the subject's legally acceptable representative/Impartial Witness, and by the person who conducted the informed consent discussion, will be retained by the Investigator. The Investigator will supply all enrolled subjects with a copy of their signed informed consent.

The consent form may need to be revised during the study should important new information become available that may be relevant to the safety of the subject. In this instance approval should always be given by the IRB/IEC and existing subjects informed of the changes and re-consented.

The subject should at their own discretion inform their primary physician about their participation in the study.

18. Data Monitoring Procedures and Quality Assurance

The Sponsor has ethical, legal, and scientific obligations to conduct this study in accordance with established research principles and ICH GCP guidelines.

18.1 Data Collection and Data Management

Study-specific data outlined in the protocol will be entered into the clinical database by individual(s) designated by the Investigator. Data will be verified electronically using a series of online programmed edit checks created by the Clinical Data Manager and programmed by the Clinical Data Programmer or Designee. Data discrepancies will be brought to the attention of the clinical team and investigated by the clinical research associate (CRA) and site staff. The CRAs will review and verify all data collected in the eCRF against source documentation, during scheduled monitoring visits. The CRA will work closely with the site staff to address any discrepancies, so that proper resolutions can be made and documented in the clinical database. An audit trail will track all changes made to the data.

18.2 Database Quality Assurance

The clinical database will be reviewed and checked for omissions, apparent errors, and values requiring further clarification, using computerized and manual procedures. Data queries requiring clarification will be generated and addressed by the investigational site. Only authorized personnel will make corrections to the clinical database, and all corrections will be documented in an audit trail.

18.3 Routine Monitoring

In order to maintain current of study progress, the Sponsor's monitors or representatives will visit the investigator sites during study conduct, in addition to maintaining telephone and written communication. Onsite visits, telephone calls, and regular inspection of the eCRFs will be conducted in order to assess subject enrolment, compliance with protocol procedures, completeness and accuracy of data entered on the eCRFs, verification of eCRF data against original source documents, and occurrence of AEs. The Investigator must provide the monitor with full access to all source and study documents.

The Investigator must agree to Sponsor-authorized personnel having direct access to the clinical (or associated) files and clinical study supplies (dispensing and storage areas) for all study subjects considered for study entry for the purpose of verifying entries made in the eCRF, and assist with their activities, if requested. Adequate time and space for CRA monitoring visits should be made available by the Investigator.

The site must complete the CRFs in a timely manner and on an ongoing basis to allow review by the CRA.

18.4 Inspections and Auditing Procedures

The Sponsor or its representative may conduct audits at the investigator sites including, but not limited to, presence of required documents, the informed consent process, and comparison of eCRFs with source documents. All medical records (progress notes) must be available for audit. The Investigator agrees to participate with audits conducted at a convenient time in a reasonable

manner.

Government regulatory authorities may also inspect the investigator site during or after the study. The Investigator or designee should contact the Sponsor/CRO immediately if this occurs. He/she must cooperate fully with regulatory authorities or other audits conducted at a convenient time in a reasonable manner.

The purpose of an audit is to assess whether ethics, regulatory, and quality requirements are fulfilled.

19. Study Management and Material

19.1 Electronic Case Report Forms

The Investigator is responsible for the quality of the data recorded in the eCRFs. The data entered should be a complete and accurate account of the subject's record collected during the study. Study data are not to be imputed directly into the eCRF but must be collected first in primary source documents at the clinical site. Completion of source documents will precede the completion of the eCRF. Source documents may be electronic, hard copy, or a combination of both. Source documents are defined as the results of original observations and activities of a clinical investigation. They include, but are not limited to, progress notes, electronic data, screening logs, telephone interviews, and recorded data from automated instruments. All source documents pertaining to this study will be maintained by the Investigators and made available for direct inspection by the authorized study personnel outlined in the ICF. The eCRF will be considered the source document for individual CRF elements such as study-specific scales if those data are collected directly onto a CRF.

Data collection will be completed according to the guidelines provided by the Sponsor or its designee in writing. All required data are to be recorded using the source documents for every subject who signed an informed consent. Site staff will be trained on the eCRF completion guidelines and requirements for source documentation.

Completed eCRFs will be reviewed by the study monitor in line with CRF review guidelines. The Sponsor or its designee will review every subject's eCRF, including source data verification for all critical data points. The source data verification plan will define the level of source data verification required for non-critical data points. Eligibility failure CRFs and source documents require only source data verification for critical data points such as informed consent, AEs, reason for termination, and inclusion/exclusion. Any discrepancies found during the CRF review will be clarified by the Investigator or designated individual. This includes on-site CRF reviews by the Sponsor or its designee, or during quality assurance review of the data.

A justification must be documented for any missing data. Any changes in the study progress notes and other source documents will be initialed and dated by a site staff member authorized to make the change. Changes will be made by striking a single line through erroneous data (so as not to obliterate the original data), and clearly entering the correct data (e.g. wrong data right data). If the reason for the change is not apparent, a brief explanation for the change will be written by the clinician in the source documentation.

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

All eCRF entries, corrections, and alterations must be made by the Investigator or designated individual. The Investigator or designated individual must adjust the eCRF (if applicable) and complete the query.

19.2 Source Documents Maintenance

The Investigator must complete a subject identification and enrolment log to allow easy identification of each subject during and after the study. This document will be reviewed by the Sponsor site contact for completeness. The subject identification and enrolment log should remain confidential and will be filed in the Investigator Site File. To ensure subject confidentiality, no copy will be made. All reports and communications relating to the study will identify subjects by assigned number only. The Investigator must also complete a subject eligibility log, which reports on all subjects who were seen to determine eligibility for inclusion in the study.

At a minimum, source documentation must be available to confirm: subject identification, eligibility, and study identification; explanation of the study and date of informed consent; dates of visits; results of safety and efficacy parameters as required by the protocol; record of all AEs; concomitant medications; date of study completion or early discontinuation, and reason for early discontinuation if applicable.

The original signed informed consent for each subject shall be filed with records kept by the Investigator and a copy shall be given to the subject

19.3 Record Maintenance

All documents pertaining to the study, including all versions of the approved study protocol, copy of the informed consent document, Health Insurance Portability and Accountability Act documents, completed eCRFs, source documents (subject records, hospital records, laboratory records, drug accountability records, etc.), and other study-related documents will be retained in the permanent archives of the study site.

The Investigator must therefore notify and obtain approval in writing from the Sponsor prior to destruction of any study records or provide an opportunity for the Sponsor to collect such records. If the Investigator withdraws from the study (e.g. relocation, retirement) all study-related records should be transferred, in a written agreement with the Sponsor, to a mutually agreed-upon designee within a Sponsor-specified timeframe.

Records must be retained in accordance with the current ICH Guidelines on GCP. All essential study documents including records of subjects, source documents, CRFs and study drug inventory must be kept on file.

Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational products. However, essential documents may be retained for a longer period if required by the applicable regulatory requirements or by agreement with the Sponsor. The Sponsor is responsible for informing the Investigator when these documents need no longer be retained.

The Investigator will not dispose of any records relevant to this study without written permission from the Sponsor and will provide the Sponsor the opportunity to collect such records. The Investigator shall take responsibility for maintaining adequate and accurate hard copy source documents of all observations and data generated during this study. Such documentation is subject to inspection by the Sponsor, its representatives and regulatory authorities.

If an Investigator moves, withdraws from an investigation or retires the responsibility for maintaining the records may be transferred to another person who will accept responsibility. Notice of transfer must be made to and agreed by the Sponsor.

19.4 Confidentiality

All information obtained during the conduct of the study with respect to the subject's state of health will be regarded as confidential. For disclosure of any such information, an agreement will be obtained in writing.

The Investigator must ensure that each subject's anonymity is maintained. On eCRFs and other documents submitted to the Sponsor or the CRO, subjects must not be identified by name. Instead, subjects will only be known by the unique subject number allocated to them in order to ensure confidentiality on all study documentation. Subjects will retain this unique number throughout the study. The Investigator will keep a separate log of these codes.

In order to comply with government regulatory guidelines and to ensure subject safety, it may be necessary for the Sponsor and its representative, the CRO personnel, the local research review board, or the US FDA to review subjects' medical records as they relate to this study. Only the subject's unique number on the CRFs will identify him/her, but their full names may be made known to a drug regulatory authority or other authorized government or health care officials, if necessary, and to personnel designated by the Sponsor.

All subject bodily fluids, and/or other materials collected shall be used solely in accordance with this protocol and the informed consent signed by the subject, unless otherwise agreed to in writing by the subject.

Documents that are not for submission to the Sponsor or the CRO will be maintained by the Investigator in strict confidence, except to the extent necessary to allow monitoring by the Sponsor and the CRO, and auditing by regulatory authorities. No documents identifying subjects by name will leave the investigator site and subject identity will remain confidential in all publications related to the study.

20. Administration requirements

20.1 Regulatory Approval

GenSight or their appointed agents will be responsible for ensuring that appropriate regulatory authority approvals are obtained, according to local country requirements.

No subject may enter the study until this approval has been obtained. A copy of the approval (where one is provided, according to local country requirements) will be provided to the Investigator and to the IRB(s)/IEC(s).

20.2 Protocol Compliance

In accordance with ICH Topic E6 (R1) Guideline for GCP the Investigator should not implement any deviation from, or changes of the protocol without agreement by the Sponsor and documented approval from the IRB/IECs of a protocol amendment, except where necessary to eliminate an immediate hazard(s) to study subjects, or when the change(s) involves only logistical or administrative aspects of the study (e.g. change in monitor(s), change of telephone number(s)).

Any change to the protocol must be handled as a protocol amendment. Any potential amendment must be approved by the Sponsor. A written amendment must be submitted to the appropriate regulatory authorities and to the IRB/IECs assuming this responsibility. The Investigator must await IRB/IEC approval of protocol amendments before implementing the changes, except where necessary to eliminate apparent immediate hazard to subjects. In these cases, the IRB/IEC must be notified within 5 business days of the change. All amendments to the protocol must be approved in writing by both the appropriate regulatory authorities and the IRB/IEC, except for administrative amendments, which require notification but not written approval. Once approved, the protocol amendment will be distributed to all recipients of the original protocol, with instructions to append the amendment to the protocol.

If, in the judgment of the local IRB/IEC, the Investigator and/or Sponsor, the protocol amendment alters the study design, procedures and/or increases the potential risk to the subject, the currently approved written ICF will require modification. The modified ICF must also be reviewed and approved by the Sponsor, appropriate regulatory authorities, and the IRB/IEC. In such cases, repeat informed consent must be obtained from subjects enrolled in the study before participation continues.

20.3 Protocol Adherence and Deviations

The protocol must be read thoroughly, and the instructions must be followed. However, exceptions will be made in emergency situations when the protection, safety, and well-being of the subject requires immediate intervention based on the judgment of the Investigator or a responsible, appropriately trained, and credentialed professional(s) designated by the Investigator as a sub-investigator.

Any protocol deviation related to the conduct of the study, subject management, or subject assessment will be documented and discussed with the Investigator on a case by case basis.

20.4 Contractual and Financial Details

The Investigator (and/or, as appropriate, the hospital administrative representative) and the Sponsor will sign a clinical study agreement prior to the start of the study, outlining overall Sponsor and Investigator responsibilities in relation to the study. The contract should describe whether costs for pharmacy, laboratory and other protocol-required services are being paid directly or indirectly.

The disclosure of any financial interests from each Investigator or sub-Investigator is required for this study, including financial interests of the spouse and each dependent child of the Investigator who is directly involved in the treatment or evaluation of research subjects that could affect the reliability of data submitted to regulatory authorities. Financial Disclosure Statements will need to be completed, as requested by FDA CFR 21 Part 54 for all US investigators; and by EU investigators if data from these sites will be used to support a New Drug Application in the US.

ICH requires that the clinical investigator make all clinical trial-related records available for direct access by the CRA, auditor, IRB, or regulatory agency (4.9.7) and that financial aspects of the trial be in the Investigator's files (8.2.4). FDA currently does not require financial records, nor mandate "direct access" (312.62).

20.5 Insurance, Indemnity and Compensation

GenSight undertakes to maintain an appropriate clinical study insurance policy. Deviations from the study protocol are not permitted and shall not be covered by the statutory subject insurance scheme.

20.6 Investigational Site File Management

The Investigator is responsible for assuring that the Investigational Site Central File is maintained. The Site Central File will contain, but will not be limited to, the information listed below:

(1) Investigator’s Brochure;
(2) Current, signed version of the protocol;
(3) Protocol amendments (if applicable);
(4) Operations Manual (if applicable);
(5) Current ICF (blank);
(6) Curricula Vitae of Investigator(s) and sub-investigator(s) and photocopy of their respective license(s) where required by law; Original US FDA Form 1572 (for all studies conducted under US IND regulations), signed by all Principal Investigators. The names of any sub-investigators must appear on this form. Investigators must also complete all regulatory documentation as required the ICH GCP and by local or national regulations;
(7) Documentation of IRB/IEC approval of the protocol, the ICF, any protocol amendments, and any ICF revisions;
(8) All correspondence between the Investigator, IRB/IEC, and the Sponsor/CRO relating to study conduct;
(9) Lab certification(s);
(10) Monitoring log;
(11) Signature list of all staff entering data in the eCRF; and
(12) Signature list of all staff completing drug accountability summaries.

20.7 Discontinuation of the Study

Upon completion of the study, study closeout activities must be conducted by the Sponsor or their designee in conjunction with the Investigator, as appropriate.

In addition, the Sponsor reserves the right to temporarily suspend or prematurely discontinue this study at any time and for any reason. If such action is taken, the Sponsor will discuss this with the Investigator (including the reason[s] for taking such action) at that time. The Sponsor will also inform the regulatory authorities of the suspension or termination of the study and the reason(s) for the action. If required by applicable regulations, the Investigator must inform the IRB/IEC promptly and provide the reason(s) for the suspension or termination. If the study is prematurely discontinued, all study data and study drug remaining on site must be returned to the Sponsor or its designee.

20.8 Clinical Study Report

A final clinical study report will be prepared according to the ICH Guideline on Structure and Contents of Clinical Study Reports. A final clinical study report will be prepared regardless of whether the study is completed or prematurely terminated. The Sponsor will provide each Investigator with a copy of the final report for retention.

20.9 Publication Policy

After completion of the study, the Investigator(s) may prepare a joint publication with the Sponsor. The Investigator(s) must undertake not to submit any part of the data from this protocol for publication without the prior consent of GenSight.

21. References

Abedi G., et al. (2013). " Incidence and Management of Elevated Intraocular Pressure with Antivascular Endothelial Growth Factor Agents" Ophthalmology 28(3):126–130.

Bainbridge JWB, Mehat MS, et al. (2015) “Long-Term Effect of Gene Therapy on Leber’s Congenital Amaurosis.” N Engl J Med 372:1887-97.

Bainbridge JWB, Smith AJ, et al. (2008) “Effect of Gene Therapy on Visual Function in Leber’s Congenital Amaurosis”. New England Journal of Medicine 358:2231-9.

Bakri, S.J., J.S. Pulido, et al. (2009). "Immediate intraocular pressure changes following intravitreal injections of triamcinolone, pegaptanib, and bevacizumab". Eye 23(1):181–185.

Balducci N, Savini G, et al. (2016) Macular nerve fibre and ganglion cell layer changes in acute Leber’s hereditary optic neuropathy. Br J Ophthalmol 100(9):1232-7.

Barboni P, Savini G, et al. (2006). “Leber's hereditary optic neuropathy with childhood onset.” Invest Ophthalmol Vis Sci 47(12):5303-5309.

Barboni P, Carbonelli M, et al. (2010) Natural History of Leber’s Hereditary Optic Neuropathy: Longitudinal Analysis of the Retinal Nerve Fiber Layer by Optical Coherence Tomography. Ophthalmology 117:623–627.

Bennett, S. J. (2003), "Immune response following intraocular delivery of recombinant viral vectors." Gene Therapy 10, 977–98.

Bennett J, Ashtari M, et al. “AAV2 Gene Therapy Readministration in Three Adults with Congenital Blindness.” Sci Transl Med 4, 120ra15; DOI: 10.1126/scitranslmed.3002865

Cideciyan AV, Hauswirth WW, et al. (2009) “Human RPE65 Gene Therapy for Leber Congenital Amaurosis: Persistence of Early Visual Improvements and Safety at 1 Year”. Human Gene Therapy 20:999–1004.

Dedania V.S., and S.J. Bakri (2015). "Sustained elevation of intraocular pressure after intravitreal anti-vegf agents What Is the Evidence?" Retina 35:841–858, 2015.

El Chehab H. et al. (2013). "Effect of topical pressure-lowering medication on prevention of intraocular pressure spikes after intravitreal injection." European J Ophthalmology 2013;23 (3):277-283.

Frenkel MPC, Haji SA, and Frenkel REP. (2010) “Effect of Prophylactic Intraocular Pressure–Lowering Medication on Intraocular Pressure Spikes After Intravitreal Injections”. Arch Ophthalmol. 128(12):1523-1527.

Guy J, Feuer WJ, et al. (2016) “LHON Gene Therapy Clinical Trial: Year 2 Results”. Abstract Number: 5086 B0316. The Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) 2016, Seattle, Washington, USA.

Hauswirth WW, Aleman TS, et al. (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 19:979–990.

Hedges TR, Gobuty M, et al. (2016) The Optical Coherence Tomographic Profile of Leber Hereditary Optic Neuropathy, Neuro-Ophthalmology, 40:3,107-112, DOI: 10.3109/01658107.2016.1173709.

Hollands, H., J. Wong, et al. (2007). "Short-term intraocular pressure changes after intravitreal injection of bevacizumab". Can J Ophthalmol 42(6):807–11.

Jabs DA, Nussenblatt RB, et al. (2005). “Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop.” Am J Ophthalmol 140(3):509-516.

Jacobson SG, Cideciyan AV, et al. (2012) “Gene Therapy for Leber Congenital Amaurosis Caused by *RPE65* Mutations Safety and Efficacy in 15 Children and Adults Followed Up to 3 Years.” Arch Ophthalmol. 130(1):9-24.

Karanjia R, Hwang TJ, Chen AF, Khoshnevis M, Chu ER, Alfredo A, et al. (2014). A Method for Quantifying Off Chart Visual Acuities. Poster 153 North American Neuro-Ophthalmology Society Annual Meeting Syllabus. 321.

Katayama B.Y., et al. (2014). " Comparison of Acetazolamide, Brimonidine, and Anterior Chamber Paracentesis for Ocular Hypertension Control After Initial Intravitreal Bevacizumab Injection: A Randomized Clinical Trial." J Glaucoma 23:461–463.

Kim G.N., et al. (2013). "Effect of Dorzolamide/Timolol or Brinzolamide/ Timolol Prophylaxis on Intravitreal Anti-VEGF Injection-Induced Intraocular Hypertension." Semin Ophthalmol 28(2):61-7.

Klopstock T, Yu-Wai-Man P, et al. (2011). “A randomized placebo controlled trial of idebenone in Leber’s hereditary optic neuropathy.” Brain 134:2677-2686.

Koilkonda RD, Guy J (2011). “Leber's Hereditary Optic Neuropathy-Gene Therapy: From Benchtop to Bedside.” J Ophthalmol 2011:179412.

Koilkonda R., H. Yu, et al. (2014). “LHON gene therapy vector prevents visual loss and optic neuropathy induced by G11778A mutant mitochondrial DNA: biodistribution and toxicology profile.” Invest Ophthalmol Vis Sci 55(12):7739-7753.

Kotterman M.A., L. Yin, et al. (2015). “Antibody neutralization poses a barrier to intravitreal adeno-associated viral vector gene delivery to non-human primates.” Gene Ther 22(2):116-126.

Kumar-Singh, K. (2008). "Barriers for retinal gene therapy: Separating fact from fiction." Vision Research 48 1671–1680.

Lam BL, Feuer WJ, et al. (2010). “Leber hereditary optic neuropathy gene therapy clinical trial recruitment: year 1.” Arch Ophthalmol 128(9):1129-1135.

Lam BL, Feuer WJ, et al. (2014). “Trial end points and natural history in patients with G11778A Leber hereditary optic neuropathy: preparation for gene therapy clinical trial.” JAMA Ophthalmol 132(4):428-436.

MacLachlan T.K. et al. (2011). “Preclinical Safety Evaluation of AAV2-sFLT01 - A Gene Therapy for Age-related Macular Degeneration.” Mol Ther. 2011 Feb;19(2):326-34.

MacLaren RE, Groppe M, et al. (2014) “Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial.” Lancet 383:1129–37.

Maguire AM, High KA, et al. (2009) “Age-dependent effects of *RPE65* gene therapy for Leber’s congenital amaurosis: a phase 1 dose-escalation trial.” Lancet 374:1597–605.

Maguire AM, Simonelli F, et al. (2008) “Safety and Efficacy of Gene Transfer for Leber’s Congenital Amaurosis.” N Engl J Med 358:2240-8.

Man PY, Turnbull DM, et al. (2002).” Leber hereditary optic neuropathy.” J Med Genet 39(3):162-169.

Mangione CM, Lee PP, et al. (2001). Development of the 25-item National Eye Institute Visual Function Questionnaire (VFQ-25). Arch Ophthalmol 119:1050-1058.

Mashima Y, Yamada K, et al. (1998). “Spectrum of pathogenic mitochondrial DNA mutations and clinical features in Japanese families with Leber's hereditary optic neuropathy.” Curr Eye Res 17(4):403-8.

Moster SJ, Moster ML, et al. (2016) Retinal Ganglion Cell and Inner Plexiform Layer Loss Correlate with Visual Acuity Loss in LHON: A Longitudinal, Segmentation OCT Analysis. Invest Ophthalmol Vis Sci. 57(8):3872-83.

Murray, C.D., D. Wood, et al. (2014). "Short-term intraocular pressure trends following intravitreal ranibizumab injections for neovascular age-related macular degeneration—the role of oral acetazolamide in protecting glaucoma patients." Eye 28, 1218–1222.

Nakamura M, Fujiwara Y, et al. (1993).” Homoplasmic and exclusive ND4 gene mutation in Japanese pedigrees with Leber's disease.” Invest Ophthalmol Vis Sci 34(3):488-495.

Newman NJ (2005a). “Hereditary optic neuropathies: from the mitochondria to the optic nerve.” Am J Ophthalmol 140(3):517-523.

Newman NJ, Biousse V. (2004). “Hereditary optic neuropathies.” Eye (Lond) 18(11):1144-1160.

Newman NJ, Biousse V, et al. (2005). “Prophylaxis for second eye involvement in Leber Hereditary Optic Neuropathy: an open-labeled, nonrandomized multicenter trial of topical brimonidine purite.” Am J Ophthalmol 140(3):407-415.

Newman NJ, Lott MT, et al. (1991). “The clinical characteristics of pedigrees of Leber's hereditary optic neuropathy with the 11778 mutation.” Am J Ophthalmol 111(6):750-762.

Nikoskelainen EK, Huoponen K, et al. (1996). "Ophthalmologic findings in Leber hereditary optic neuropathy, with special reference to mtDNA mutations." Ophthalmology 103(3):504-514.

Nussenblatt RB, Palestine AG, et al (1985). "Standardization of vitreal inflammatory activity in intermediate and posterior uveitis." Ophthalmology 92(4):467-471.

Polman CH, Reingold SC, et al. (2011) Diagnostic Criteria for Multiple Sclerosis: 2010 Revisions to the McDonald Criteria. Ann Neurol 69:292–302.

Puomila A, Hämäläinen P, et al. (2007). "Epidemiology and penetrance of Leber hereditary optic neuropathy in Finland." Eur J Hum Genet 15(10):1079-1089.

Rakoczy EP, Lai CM, et al. (2015) "Gene therapy with recombinant adeno-associated vectors for neovascular age-related macular degeneration: 1 year follow-up of a phase 1 randomised clinical trial." Lancet 386: 2395–403.

Ramachandran P.S., and V. Lee, (2016) "Evaluation of Dose and Safety of AAV7m8 and AAV8BP2 in the Non-Human Primate Retina." Hum Gene Ther doi:10.1089/hum.2016.111.

Raxone® European Product Assessment Report 2015. http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/003834/human_med_001900.jsp&mid=WC0b01ac058001d124 (accessed 18 May 2017).

Riordan-Eva P, Sanders MD, et al. (1995). "The clinical features of Leber's hereditary optic neuropathy defined by the presence of a pathogenic mitochondrial DNA mutation." Brain 118 (Pt 2):319-337

Sadun AA, La Morgia C, et al. (2011). "Leber's Hereditary Optic Neuropathy." Curr Treat Options Neurol 13(1):109-117.

Spruijt L, Smeets HJ, et al (2007). "A MELAS-associated ND1 mutation causing Leber Hereditary Optic neuropathy and spastic dystonia." Arch Neurol 64(6):890-3.

van Senus AH (1962). "An investigation of the occurrence of Leber's disease in the Netherlands." Ophthalmologica 144:415-419.

Wallace DC, Singh G, et al. (1988). "Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy." Science 242(4884):1427-1430.

Ware JE, Kosinski M, et al. (2007). User's Manual for the SF-36v2™ Health Survey (2nd Ed). Lincoln, RI, QualityMetric Incorporated.

Wan X., et al. (2016). "Efficacy and Safety of rAAV2-ND4 Treatment for Leber's Hereditary Optic Neuropathy." Sci Rep 6:21587. doi: 10.1038/srep21587.

Wu, H., and T.C. Chen. (2009). "The Effects of Intravitreal Ophthalmic Medications on Intraocular Pressure." Seminars in Ophthalmology 24:100–105.

Yang S, Ma S, et al. (2016) "Long-term outcomes of gene therapy for the treatment of Leber's hereditary optic neuropathy". EBioMedicine 10:258–268.

Ye G.J., E. Budzynski, et al. (2015) “Safety and Biodistribution Evaluation in Cynomolgus Macaques of rAAV2tYF-CB-hRS1, a Recombinant Adeno-Associated Virus Vector Expressing Retinoschisis.” Hum Gene Ther Clin Dev 26(3):165-76.

Yu-Wai-Man P, Griffiths PG, et al. (2009). “Inherited mitochondrial optic neuropathies.” J Med Genet 46(3):145-158.

Yu-Wai-Man P, Griffiths PG, et al. (2011). “Mitochondrial optic neuropathies - disease mechanisms and therapeutic strategies.” Prog Retin Eye Res 30(2):81-114.

22. APPENDICES

22.1 APPENDIX I – Names of Study Personnel

Sponsor:	GenSight Biologics SA 74 rue du Faubourg Saint-Antoine 75012 Paris France
Medical Monitor:	Magali Taiel, MD GenSight Biologics (Sponsor) 74 rue du Faubourg Saint-Antoine 75012 Paris France
International Clinical Project Manager:	[redacted] GenSight Biologics (Sponsor) 74 rue du Faubourg Saint-Antoine 75012 Paris France
Clinical Research Organization:	IQVIA Ltd. Eastpoint Business Park Estuary House North Dock, Fairview, Dublin, D03 Y6A2 Ireland
Central Laboratory:	Q2 Solutions 5827 South Miami Blvd Morrisville, NC 27560 USA
Central Ophthalmic Assessment Reader:	Annesley Eye Brain Center (AEBC) 1304 Society Drive Claymont, DE 19703 USA
Pharmacovigilance Activities Vendor:	Voisin Consulting Life Sciences 3 rue des Longs Prés 92100 Boulogne-Billancourt France
Interactive Response System (IRS)	Cenduit 4825 Creekstone Drive, Suite 400 Durham, NC 27703 USA