COVER PAGE

Official Title:	A Dose Escalation (Phase 1), and Dose Expansion (Phase 2/3) Clinical Trial of Retinal Gene Therapy for X-Linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)
NCT Number:	NCT03116113
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Statistical Analysis Plan

A Dose Escalation (Phase 1), and a Dose Expansion (Phase 2/3) Clinical Trial of Retinal Gene Therapy for X-Linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)

XIRIUS Study

Protocol Number:	**274RP101(NSR-RPGR-01) / NCT03116113**
EudraCT Number:	**2016-003852-60**
Investigational Medicinal Product:	**AAV8-RPGR**

Document Status:	FINAL
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2 SUMMARY OF CHANGES

Version 1.0 of the XIRIUS Statistical Analysis Plan (dated 21 March 2019) was aligned with the XIRIUS protocol up to version 8.1. Version 2.0 of the SAP was created to align with version 9.0 of the XIRIUS protocol, dated 14 August 2019. These changes are summarized below.

- The primary efficacy endpoint (originally the primary efficacy endpoint is the proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of the 16 central loci in microperimetry at 12 months) was clarified as specifying that the 10-2 grid is used in performing the MAIA microperimetry test.
- Key secondary efficacy endpoints have been eliminated based on protocol assistance received on 18 June 2019 from the European Medicines Agency.
- [redacted]
- [redacted]
- The outcomes-assessor masking of assessments was clarified for Part II.
- The methodology of the Part II 3-month interim analysis assisted by the Data Monitoring Committee, and the masked and unmasked operational teams are fully described.
- Based on feedback from FDA after submission of version 1.0 of the XIRIUS SAP and a teleconference of 23 May 2019, the primary efficacy analysis population has been changed to the Intent to Treat (ITT) population and not the modified ITT study population, which is now used for sensitivity and supportive analyses only.
- Handling of missing values has been clarified for continuous and categorical endpoints.
- Statistical analyses of the categorical visual acuity endpoints have been clarified for Part II.
- Adaptive-optics optical coherence tomography has been removed from the SAP and protocol.
- Table 2, the Schedule of Study Procedures, has been updated to align with version 9.0 of the XIRIUS protocol.

At that time when protocol V10.0 was amended, SAP was updated accordingly. For SAP version 3.0 it aligns with XIRIUS protocol V11.0, dated 1 Oct 2020. The changes are summarized as below:

- The sample size section was updated due to study drug availability. The power was updated to ensure the study is adequately powered with type I error
- Multiplicity adjustment has been added for testing two doses for the primary endpoint

- Instructions are now provided for missed final visits due to COVID-19, clarifying that observations collected 6 months outside the last visit window will be included in analysis
- Interim analysis description has been updated as it may be implemented, but it is not a requirement
- The definition of the Safety Analysis Set in Part II has been updated
- mITT Analysis Set has been removed

The missing data imputation approaches have been updated for the primary endpoint and selected efficacy endpointsSAP Version 3.0 was amended to Version 4.0, dated on 10March 2021. The changes are summarized below:

- A protocol deviation was identified at one site which involved the use of unmasked assessors (instead of the protocol-prescribed masked assessors) conducting microperimetry, best-corrected visual acuity, low luminance visual acuity, contrast sensitivity [redacted] at the scheduled visits from Month 3 to Month 12. Sensitivity analyses are added to evaluate the impact of this deviation on these data

- Strategies for examining and excluding extreme outliers for mean sensitivity are added

- [redacted]

- Fisher's Exact Boschloo test are implemented for LLVA and BCVA change from baseline, only when the improvement is ≥10, ≥15 letters from baseline in Part II at Month 12

APPROVAL

This document has been reviewed and approved by:		
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3 ABBREVIATIONS

Abbreviation	Term
AAV	Adeno-Associated Viral Vector
AAV8-RPGR	adeno-associated virus serotype 8-retinitis pigmentosa GTP hydrolase regulator AAV8 virus particle encapsulating cDNA for the coRPGR gene
AE	Adverse Event
AF	AutoFluorescence
ANCOVA	Analysis of Covariance
ATC	Anatomical Therapeutic Chemical
ADA	Anti-Drug Antibody
BCVA	Best Corrected Visual Acuity
BLQ	Below level of quantification
CI	Confidence Interval
CRF	Case Report Form
CSS	Contrast Sensitivity Score
CTCAE	Common Terminology Criteria for Adverse Event
CTL	Cytotoxic T lymphocytes
COVID-19	Coronavirus Disease 2019
dB	decibel
IDMC	Independent Data Monitoring Committee
DLT	Dose-Limiting Toxicity
eCRF	electronic Case Report Form
ERM	Epiretinal Membrane
ELISpot	Enzyme-Linked Immunospot
ET	Early Termination
ETDRS	Early Treatment Diabetic Retinopathy Study
EZ	Ellipsoid zone
FAF	Fundus autofluorescence
FC	Foveal Center
IOP	Intraocular Pressure
IQR	Interquartile Range
ITT	Intention-to-treat
LLVA	Low Luminance Visual Acuity
LOCF	Last observation carried forward
LS	Least square
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple Imputation
[redacted]	[redacted]
MMRM	Mixed model repeated measure
MTD	Maximum Tolerated Dose
NAb	Neutralizing Antibody
OD	Oculus Dextrus (Right Eye)
OU	Oculus Uterque (Both Eyes)
OS	Oculus Sinister (Left Eye)
PBMCs	Peripheral Blood Mononuclear Cells
PD	Protocol Deviation
RF	Reliability Factor

PT	Preferred Term
Q1	Quartile 1
Q3	Quartile 3
RPGR	Retinitis Pigmentosa GTPase Regulator
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SD	Standard Deviation
SD-OCT	Spectral Domain Optical Coherence Tomography
SE1/SE2	study eye 1/study eye 2
SFU	Spot forming units
SOC	System Organ Class
SRF	Subretinal Fluid
TEAE	Treatment Emergent Adverse Event
TOF	Targeted Ocular Findings
VA	Visual Acuity
VMT	Vitreomacular Traction
[redacted]	[redacted]
vg	Vector genomes (equivalent to genome copies)
WHODRUG	World Health Organization Drug Dictionary
XLRP	X-linked Retinitis Pigmentosa

4 INTRODUCTION

This document presents the statistical analysis plan (SAP) for Biogen, Protocol No. 274RP101(formerly NSR-RPGR-01): A Dose Escalation (Phase 1), and Dose Expansion (Phase 2/3) Clinical Trial of Retinal Gene Therapy for X-Linked Retinitis Pigmentosa (XLRP) Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR) (XIRIUS Study).

This analysis plan is based on the protocol amendment V11 dated 1 Oct 2020; incorporating amendments no. 1, dated 01DEC2016 to amendment no. 11.0, dated 1 Oct 2020.

The SAP provides the description of the final analyses for the generation of the clinical study report.

5 STUDY OBJECTIVES AND ENDPOINTS

5.1 Objective

The objective of the study is to evaluate the safety, tolerability and efficacy of a single sub-retinal injection of AAV8-RPGR in subjects with XLRP.

5.2 Endpoints

The endpoints in the protocol are listed in a general way. To facilitate statistical analysis plan for each endpoint, more details are provided in this section.

5.2.1 Part I

5.2.1.1 Primary Endpoints

The primary safety endpoints are the incidence of dose-limiting toxicities (DLTs), and treatment-emergent adverse events (TEAEs) over a 24-month period.

5.2.1.2 Secondary and Exploratory Endpoints

- Change from baseline in microperimetry
  - Proportion of study eyes with ≥7 dB improvement from baseline at ≥5 out of the 16 central loci in microperimetry at 1, 3, 6, 9, 12, 18 and 24 months, respectively
  - Proportion of study eyes with ≥7 dB improvement from baseline at ≥5 out of the 68 loci in microperimetry at 1, 3, 6, 9, 12, 18 and 24 months, respectively
  - Change from baseline in Mean Sensitivity of the 16 central loci at 1, 3, 6, 9, 12, 18 and 24 months, respectively
  - Change from baseline in Mean Sensitivity of the 68 central loci at 1, 3, 6, 9, 12, 18 and 24 months, respectively
- Change from baseline in best-corrected visual acuity (BCVA) and Low luminance visual acuity (LLVA)
  - Change from baseline in BCVA at 1, 3, 6, 9, 12, 18 and 24 months, respectively
  - Change from baseline in LLVA at 1, 3, 6, 9, 12, 18 and 24 months, respectively

- Proportion of eyes with a ≥15 letters increase from baseline for BCVA and LLVA at 1, 3, 6, 9, 12, 18 and 24 months, respectively
- Proportion of eyes with a ≥10 letters increase from baseline for BCVA and LLVA at 1, 3, 6, 9, 12, 18 and 24 months, respectively
- Proportion of eyes with a ≥5 letters increase from baseline for BCVA and LLVA at 1, 3, 6, 9, 12, 18 and 24 months, respectively
- Proportion of eyes with a ≥15 letters loss from baseline for BCVA and LLVA at 1, 3, 6, 9, 12, 18 and 24 months, respectively
- Proportion of eyes with a ≥10 letters loss from baseline for BCVA and LLVA at 1, 3, 6, 9, 12, 18 and 24 months, respectively
- Proportion of eyes with a ≥5 letters loss from baseline for BCVA and LLVA at 1, 3, 6, 9, 12, 18 and 24 months, respectively
- Proportion of eyes with change from baseline > -5 letters for BCVA and LLVA at 1, 3, 6, 9, 12, 18 and 24 months, respectively

- Change from baseline in spectral domain optical coherence tomography (SD-OCT)
  - Change from baseline in Central Ellipsoid Area at 1, 3, 6, 9, 12, 18 and 24 months, respectively
  - Change from baseline in Central Horizontal Ellipsoid Width at 1, 3, 6, 9, 12, 18 and 24 months, respectively
- Change from baseline in Fundus Autofluorescence at 1, 3, 6, 12, 18 and 24 months
- Change from baseline in other anatomical and functional outcomes at 1, 3, 6, 9, 12, 18 and 24 months, respectively

5.2.2 Part II

5.2.2.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the proportion of study eyes with ≥7 dB improvement from baseline at ≥ 5 of the 16 central loci of the 10-2 grid assessed by Macular Integrity Assessment (MAIA) microperimetry at 12 months.

5.2.2.2 Safety Endpoint

The primary safety endpoint is the incidence of TEAEs over a 12-month period.

5.2.2.3 Secondary Endpoints

- Change from baseline in microperimetry
  - Proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of the 16 central loci in microperimetry at 1, 2, 3, 6 and 9 months, respectively
  - Proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of the 68 loci in microperimetry at 1, 2, 3, 6, 9 and 12 months, respectively
  - Change from baseline in Mean Sensitivity of the 16 central loci at 1, 2, 3, 6, 9 and 12 months, respectively
  - Change from baseline in Mean Sensitivity of the 68 loci at 1, 2, 3, 6, 9, and 12 months, respectively

- Change from baseline in BCVA and LLVA
  - Change from baseline in BCVA and LLVA at 1, 2, 3, 6, 9, and 12 months, respectively
  - Proportion of eyes with a ≥15 letter increase from baseline at 1, 2, 3, 6, 9, and 12 months, respectively
  - Proportion of eyes with a ≥10 letter increase from baseline at 1, 2, 3, 6, 9, and 12 months, respectively
  - Proportion of eyes with a ≥5 letter increase from baseline at 1, 2, 3, 6, 9, and 12 months, respectively
  - Proportion of eyes with a ≥15 letters loss from baseline for BCVA and LLVA at 1, 2, 3, 6, 9, and 12 months, respectively
  - Proportion of eyes with a ≥10 letters loss from baseline for BCVA and LLVA at 1, 2, 3, 6, 9, and 12 months, respectively
  - Proportion of eyes with a ≥5 letters loss from baseline for BCVA and LLVA at 1, 2, 3, 6, 9, and 12 months, respectively
  - Proportion of eyes with change from baseline > -5 letters for BCVA and LLVA at 1, 2, 3, 6, 9, and 12 months, respectively
- Change from baseline in visual field assessed by Octopus 900 at 3, 6 and 12 months, respectively

5.2.2.4 Exploratory Endpoints

- [redacted]
- [redacted]
  - [redacted]
  - [redacted]
- [redacted]
- [redacted]
- [redacted]

6 STUDY DESIGN

6.1 Discussion of Study Design

This is a Phase 1/2/3, first-in-human, multi-center, dose-escalation interventional study of AAV8-RPGR in male subjects with genetically confirmed XLRP. The study will be conducted in 2 parts: Part I is a dose escalation study, Part II is a dose-expansion study, with 2 doses selected from Part I based on safety and efficacy, and a third untreated group to allow for a controlled comparison of efficacy and safety.

Part I will identify the maximum tolerated dose (MTD) using a dose-escalation scheme. Part II will expand 2 doses, allowing for a broader assessment of the safety and efficacy of AAV8-RPGR with a larger sample size, including up to approximately 30 subjects randomized 1:1:1 to a high-dose, a low-dose, and an untreated arm. Part I primarily evaluates safety, defined by incidence of Dose Limiting Toxicities (DLTs) and TEAEs over a 24-month period. Phase II evaluates safety and efficacy, with inclusion of a primary efficacy endpoint, improvement from baseline in microperimetry evaluated at 12 months, and safety and secondary efficacy evaluated at 1, 2, 3, 6, 9, and 12 months post-treatment. An administrative interim analysis may be conducted during follow-up of Part II.

Part I

Part I will use a 3+3 escalation scheme for administration of AAV8-RPGR.

Part I of the study will involve up to 6 dose cohorts, with AAV8-RPGR doses of 5×10^9 vg (Cohort 1), 1×10^10 vg (Cohort 2), 5×10^10 vg (Cohort 3), 1×10^11 vg (Cohort 4), 2.5×10^11 vg (Cohort 5), and 5×10^11 vg (Cohort 6). Each eligible subject will receive AAV8-RPGR in their study eye and be monitored for DLTs.

An Independent Data Monitoring Committee (IDMC) will be used to review safety data before confirming whether escalation to a higher dose level can occur. There is a potential for surgical complications resulting in safety events that meet the criteria for a DLT. In such cases, the IDMC will make the final adjudication as to whether the event is a DLT.

The IDMC will review safety data for each cohort when at least 3 subjects have been dosed at a particular level. However, if 2 subjects within a cohort have a DLT(s), dosing will not proceed to subsequent subjects until safety data are reviewed by the IDMC.

Three to 6 subjects are planned per dose cohort; however, the actual number of subjects enrolled into each cohort will depend on the toxicity observed. If no DLTs are observed in the first 3 subjects treated within a cohort, then escalation to the next dose cohort can proceed. If 1 DLT is reported within a 3-subject cohort, an additional 3 subjects will be added and treated at the same dose level. If there are no further DLTs reported in the additional 3 subjects, then escalation to the next dose cohort can proceed. If ≥2 subjects within a cohort (3 or 6 subjects) have a DLT(s), then the MTD will be identified as the previous (lower) dose. Dosing will cease under this protocol and further investigation may occur following a protocol amendment.

Part II

Approximately 30 additional subjects will be randomized in a 1:1:1 allocation ratio to a high-dose group (2.5×10^11 vg), a low-dose group (5×10^10 vg), and an untreated control group.

Study data will be collected for both eyes of each subject. Since treatment requires an invasive surgical procedure under general anaesthesia, the sponsor, investigator and subject will be unmasked to the study procedure (i.e., vitrectomy and sub-retinal injection). However within the treated groups, the sponsor, investigator and subject will be masked to the assigned dose. To further minimise potential bias of the treated and non-treated eye evaluations, all subjective ophthalmic assessments at the Screening/Baseline Visit (Visit 1) and from Month 3 (Visit 6) onwards will be conducted by an assessor who is masked to which eye has received treatment and to which group the subject has been assigned to.

As a note, Version 6 (dated 18MAY18) of the protocol introduced the dose expansion phase of the study, where subjects were to be randomized in 2 groups (i.e., high dose and low dose) in a 2:1 allocation ratio. As of 07 November 2018, 3 subjects were randomized under the 2:1 allocation ratio as per Version 6 (prior to Version 8 finalization).

6.2 Study Treatment

Part I, Dose Escalation: all subjects will undergo vitrectomy and retinal detachment in their study eye and then receive a single, sub-retinal injection of AAV8-RPGR. Subjects will receive an AAV8-RPGR dose of 5×10^9 vg (Cohort 1), 1×10^10 vg (Cohort 2), 5×10^10 vg (Cohort 3), 1×10^11 vg (Cohort 4), 2.5×10^11 vg (Cohort 5), or 5×10^11 vg (Cohort 6).

The layout of the summary table is:

Cohort 1 5×10^9 vg	Cohort 2 1×10^10 vg	Cohort 3 5×10^10 vg	Cohort 4 1×10^11 vg	Cohort 5 2.5×10^11 vg	Cohort 6 5×10^11 vg	Cohorts 3-6	Part I Total

For ocular inflammation-related and visual acuity-related tables, the cohorts of 1-3, and 4-6 will be displayed.

Cohort 1 5×10^9 vg	Cohort 2 1×10^10 vg	Cohort 3 5×10^10 vg	Cohort 4 1×10^11 vg	Cohort 5 2.5×10^11 vg	Cohort 6 5×10^11 vg	Cohort 1-3	Cohorts 4-6	Part I Total

Part II, Dose Expansion: subjects will be assigned to one of the following: high-dose (2.5×10^11 vg), low-dose (5×10^10 vg), or an untreated control arm.

The efficacy summary table will be displayed as:

High Dose 2.5×10^11 vg	Low Dose 5×10^10 vg	Part II Treated	Part II Untreated

The safety summary table will be displayed as:

High Dose 2.5×10^11 vg	Low Dose 5×10^10 vg	Part II Treated	Part II Untreated	Part I and II All Treated Doses

6.3 Study Schedule

Part I will consist of 11 visits over a 24-month evaluation period. Part II consists of 10 visits over a 12-month period. At the Screening/Baseline Visit, each subject will be assessed for eligibility of both eyes. Only 1 eye will receive treatment (the “study eye”), and the untreated eye will be designated as the “fellow eye”. Selection of the “study eye” will be made on clinical grounds and will generally be the worse eye affected.

Subjects will be assessed for safety and efficacy throughout the study as indicated in the Schedule of Study Procedures (refer to the protocol).

Subjects who develop cataracts may undergo cataract surgery if deemed clinically necessary; if surgery is performed, it should be carried out at least 4 weeks before their final visit.

The end of the trial is the date the last subject completes his final-visit assessments (or early termination [ET] assessments in the event of premature discontinuation) or the date of last data collection if the last subject is lost to follow-up.

6.4 Randomization

Part I, the dose-escalation portion of this study, is not randomized.

In Part II, after the study eye is assigned, subjects will be randomized to 1 of 3 groups with a 1:1:1 allocation ratio: (1) treatment with AAV8-RPGR at a high dose (2.5×10^11 vg); (2) treatment with AAV8-RPGR at a low dose (5×10^10 vg); (3) an untreated control group. Randomization will be generated using a validated system that automates the random assignment to treatment groups. Once a subject is deemed eligible, the investigative site (or authorized designee) will access the system, and the subject will be randomized using a standard randomization procedure.

In Version 6 of the protocol 3 subjects were randomized in 2 groups to high dose and low dose in a 2:1 allocation ratio.

6.5 Masking

Part I of the study is open-label.

Part II is double-masked (sponsor, investigator/site team, and subject will be masked) to the assigned dose, and unmasked with respect to the treatment administration.

In Part II, it is preferable that the same assessor who performed the efficacy endpoint assessments at Screening/Baseline also perform the assessments during the masked period from Month 3 to Month 12. For the immediate post-operative visits, masking of the assessors will not be viable as clinical signs of surgery will be apparent (i.e. redness, swelling). Therefore, unmasked assessors will perform all ophthalmic assessments at Visit 3 (Day 1) and Visit 4 (Day 7). For Visit 6 (Month 3) onwards, masked assessors will be used, as any signs of surgery will have dissipated and it will not be possible clinically to differentiate between those subjects that have not undergone surgery, and those subjects that have undergone surgery and received active treatment. The following assessments will be masked at 3, 6, 9 and 12 months: BCVA, LLVA, microperimetry, contrast sensitivity, and the [redacted].

Subjects randomized to the untreated control group will not be required to attend the site at Visit 2, 3 or 4. As the key purpose of Visit 2 is surgery, and Visit 3 and 4 are post-operative safety, there is limited utility in control subjects attending. Therefore, to limit the study burden for control subjects, thereby potentially reducing the risk of subject withdrawal at this stage and reducing the possibility of further unmasking due to direct contact and communication with fellow participants, control subjects are not scheduled to attend the clinic for study visits at these times.

In order to minimize bias further, masked assessors will not have access to the subject's medical records, source documentation or eCRF as data entries or notation (such as use of peri-operative corticosteroid) may be sources of unmasking. From Visit 6 (Month 3) onwards, the masked assessor will also read a pre-written statement to each subject, regardless of randomization, reminding them of the masked nature of the study, and to avoid any reference to prior surgery/non-surgery, which eye may have received treatment or to allude to any information that may unmask the assessor as to which group the subject has been assigned to.

Furthermore, it is anticipated that a subset of the subjects participating in the trial will be active on social media. Following appropriate approval by the Independent Ethics Committee (IEC)/Institutional Review Board (IRB), the patient informed consent form will

request that subjects refrain from posting any details of study participation on social media, which may unmask the assessors to the group the subject has been assigned to. This request will be reiterated at subject visits by the investigator and within the pre-written statement.

Subjects randomized to the AAV8-RPGR treatment groups, surgeons, the investigative team and the study sponsor will be masked to which dose of AAV8-RPGR the subject has been assigned to. Unmasked study site personnel will be assigned the responsibility of performing dilution, which will take place in a designated area remote from the investigative team to preserve masking of the treatment arm. Personnel delegated to perform the dilution will not be involved in any other aspect of the study (i.e., consent, safety/efficacy assessments, surgical procedure).

During Part II, an administrative IA may be performed. In the event that an IA is conducted, the study will continue as planned in a masked fashion.

6.6 Sample Size

Due to the nature of the study design of Part I, no formal sample size computation was performed.

In Part II, the primary endpoint is the proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of the 16 central loci of the 10-2 grid assessed by MAIA microperimetry at 12 months. A sample size of 10 subjects from a treatment group (either high dose or low dose) and 9 subjects from the untreated control group, will provide approximately 87% power, at a right-sided significance level of 0.10 assuming the treatment group has a 50% response rate and the untreated group has a 5% response rate. The power calculation is based on Fisher's Exact-Boschloo test with a Berger-Boos correction of beta = 0.001.

7 STATISTICAL METHODOLOGY

7.1 Analysis Sets

Part I

The Safety Analysis Set will consist of all subjects who receive study treatment (vitrectomy/AAV8-RPGR). The Safety Analysis Set will be used for all analyses in Part I.

Part II

The Safety Analysis Set will consist of all subjects who are randomized, under both the 2- and the 3-arm randomization schedules and receive study treatment when randomized to active treatment, or attend Visit 2 (telephone call) when randomized to control (no treatment). The Safety Analysis Set will be used for safety analyses in Part II. Subjects will be analyzed based on the actual treatment they received.

The Intent-to-Treat (ITT) Analysis Set will consist of all subjects who are randomized, under the 3-arm randomization schedules. The ITT Analysis Set will be used for efficacy analyses in Part II. Subjects will be analyzed based on the treatment to which they were randomized.

7.2 Conventions

Summary statistics will be presented for both eyes (study eye versus fellow eye). In Part I, no formal statistical comparison will be performed. In Part II, formal statistical testing will be performed.

Continuous variables and their change from baseline will be summarized using descriptive statistics (number of subjects[n], mean, standard deviation, median, minimum and maximum, first and third quartiles, fifth and ninety-fifth percentiles). The confidence intervals (CIs) of the mean and the mean change from baseline at each visit may be provided where applicable. If CI is reported, the CI of the mean value is calculated by one sample t-test, and the CI of mean change from baseline is calculated by paired t-test. For categorical variables, the number and proportion of subjects pertaining to each category will be presented. The CIs of the proportion may be provided where applicable. In the event of CI of the proportion is reported, it is calculated by the Clopper-Pearson method.

Age is calculated as (Informed consent date – date of birth + 1)/365.25 and displayed as an integer. If the day and month of birth are not known, the date of birth will be imputed as 01July of the birth year.

7.3 Time Points and Visit Windows

The study baseline is defined as the most recent non-missing measurement (scheduled or unscheduled) prior to the study treatment. Where this value is missing or unavailable, then an available Visit 2 (prior to treatment) result will be used.

Change from baseline is defined as the difference between an assessment at the considered visit and the baseline.

Study Day 0: the date of the surgery

Study Day is calculated as:

- For a date on or after Study Day 0
- Study Day = (Date of Interest) – (Study Day 0) + 1
- For a date before Study Day 0
- Study Day = (Date of Interest) – (Study Day 0)

All data will be analysed using nominal study visits as defined in the Study Schedule and eCRF. However if the study visits from Month 24 for Part I and Month 12 for Part II are collected within 6 months out of window, these visits will be included in the analysis.

7.4 Interim Analysis

Part I

[redacted] A final analysis will be conducted after all subjects complete Part I.

Part II

In Part II, an administrative IA may be performed. If an IA is performed, the study will continue as planned in a masked fashion.

7.5 Adjustment for Multiplicity

Part I

This is the dose-escalation part of the study to identify the MTD based on the evaluation of the benefit-risk without any formal statistical comparison. Adjustment for multiplicity is, therefore not applicable.

Part II

The primary efficacy endpoint of Part II is defined as the proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of the 16 central loci of the 10-2 grid assessed by MAIA microperimetry at 12 months.

At final analysis, the primary endpoint will be tested byHochberg's (Hochberg 1988) step-up method with familywise error rate controlled at right-sided 0.10. The high dose vs. control group, and the low dose vs. control group will be tested respectively. The study will be declared positive if either dose or both doses achieve statistical significance.

Two hypotheses will be tested for primary endpoint:

High dose vs control group:

H_0: $p_{trt} = p_{untr}$ versus H_1: $p_{trt} > p_{untr}$

Low dose vs control group:

H_0: $p_{trt} = p_{untr}$ versus H_1: $p_{trt} > p_{untr}$

The algorithm is based on data-driven ordering of hypotheses $H_{(1)}$ and $H_{(2)}$ corresponding to the ordered p-values $P_{(1)} < P_{(2)}$.

Step 1: start with larger p-value $P_{(2)}$. If $P_{(2)} < 0.10$ (one-sided) then reject null hypotheses and claim this test is significant. The remaining hypothesis test is also rejected. Both doses achieve statistical significance. Otherwise if $P_{(2)} \geq 0.10$ this is not significant and proceed to the next test to compare $P_{(1)}$ with 0.05 (one-sided).

Step 2: if $P_{(1)} < 0.05$ (one-sided) then testing stops. The dose associated with $P_{(1)}$ is claimed statistically significant than control group. The dose associated with $P_{(2)}$ is not statistically significant. Or if $P_{(1)} \geq 0.05$ (one-sided) then testing stops and both doses are not statistically significant.

7.5 Imputation of Partial Dates for Adverse Events and Concomitant Medication

For on-study adverse events and concomitant medications with partially missing dates, the imputation rule is tabulated as follows.

	Missing	Condition	Imputed Value
Start date (AE, concomitant medication)	Day	The event started in the same year and month as Study Day 1 (or treatment period start date)	Study Day 1 (or treatment period start date)
		Otherwise	01
	Day and month	The event started in the same year as Study Day 1 (or treatment period start date)	Study Day 1 (or treatment period start date)

		Otherwise	01JAN
	Completely missing (missing day, month, and year)		No imputation will be performed
Stop date (AE, concomitant medication)	Day	The event stopped in the same year and month as the End of Study date	The End of Study date or the analysis data cut-off date
		Otherwise	The last day of the month
	Day and month	The event stopped in the same year as the End of Study date	The End of Study date or the analysis data cut-off date
		Otherwise	31DEC
	Completely missing (missing day, month, and year)		No imputation will be performed

7.6 Handling of Missing Data and Extreme Outliers

The number, pattern and timing of missing data will be examined along with the reasons for withdrawal or for missing data.

All reasonable efforts will be made to obtain complete data for both eyes on all subjects. However misisng observations may occur. In addition, due to the impact of coronavirus disease 2019 (COVID-19) on the ongoing trial, some assessments might be missed or delayed.

In Part I, although the visit will still be considered out-of-window, if subjects are unable to attend the 24-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. This will be considered their 24-month visit. For efficacy endpoints, observations collected within 6 months of the last visit window will be included in analysis. Safety endpoints will be analyzed on observed data and no imputation will be performed. All analysis in Part I will be generated by descriptive statistics.

In Part II, although the visit will still be considered out-of-window, if subjects are unable to attend the 12-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. This will be considered their 12-month visit. For efficacy endpoints, observations collected within 6 months of the last visit window will be included in analysis. Additional imputation approaches for the primary endpoint and selected secondary endpoints will be also implemented.

Safety analyses will be descriptive only and will be performed using the ‘observed case’ (OC) method. No missing data imputation will be applied to safety analyses.

The 3*IQR rule method will be used to identify the extreme outliers for the mean sensitivity. A data point is an extreme outlier if it is more than 3*IQR above the third quartile (Q3) or below the first quartile (Q1) where IQR, the interquartile range, is calculated as the absolute difference between Q3 and Q1. The influence of the extreme outliers will be investigated. The extreme outliers may be excluded from the analysis based on statistical and clinical evaluation. See section 7.12.2 on handling patients with data identified with extreme outliers in mean sensitivity analysis.

7.7 Disposition of Subjects

Part I

Accounting of subjects will include number of subjects in Safety Analysis Set, number (%) of subjects who attended each visit, number (%) of subjects who completed the study, and number (%) of subjects who discontinued from study. For subjects who discontinued from study early, the reasons for discontinuation will be summarized.

Part II

Accounting of subjects will include number of subjects randomized, number of subjects in Safety Analysis Set and ITT Analysis Set, number (%) of subjects who attend each visit, number (%) of subjects who completed the study, and number (%) of subjects who discontinued from study. For subjects who discontinued from study, the reasons for discontinuation will be summarized.

7.8 Demographic and Baseline Characteristics

Demographic of age, gender, ethnicity, race will be summarized by each treatment group in the Safety Analysis Set.

Baseline ocular characteristics including mean sensitivity, LLVA, BCVA, fundus autofluorescence (FAF), [redacted], central ellipsoid area, central horizontal ellipsoid width, intraocular pressure, and Pelli-Robson contrast sensitivity will be summarized by treatment groups (except [redacted]), visits and eyes in the Safety Analysis Set. Listings for demographics and baseline characteristics will be provided.

7.9 Medical and Ocular History

Medical and ocular history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 23.1.

Ocular history will be summarized by System Organ Class (SOC) and Preferred Term (PT) under each treatment group in the Safety Analysis Set.

Where data are recorded as ‘OU’ (both eyes), the event will be attributed to each eye (study eye and fellow eye) in the summary table.

A listing of medical history will be generated.

7.10 Prior and Concomitant Medication

Part I and Part II

Prior medications are those that start and stop before the surgery. For control subjects, prior medications are defined as those with start and stop prior to Visit 2 (Day 0). Concomitant medications are all medications taken from the day of surgery, including those started before but ongoing at the time of the surgery (or Visit 2, Day 0 in the case of control subjects).

Prior and concomitant medications will be coded according to World Health Organization Drug Dictionary (WHODRUG) version B3 202009.

Prior and concomitant ocular medications with respect to site of administration will be presented by treatment groups, Anatomical Therapeutic Chemical (ATC) Level 4, PT and routes in the Safety Analysis Set. The ocular routes for site of administration include ophthalmic, intravitreal, subtenon, subconjunctival and potentially other peri-ocular routes. Ocular medications will be presented by study eye and fellow eye separately. Where an ocular medication is recorded as 'OU', it will be counted once for each eye (study eye and fellow eye) in the summary table.

Prior and concomitant non-ocular medication will be also presented by treatment groups, ATC Level 4, PT and routes in the Safety Analysis Set.

A subject with more than one occurrence of the same medication in a particular ATC class will be counted only once.

The oral corticosteroid treatment and tapering will be listed.

7.11 Exposure

As the treatment consists of a single injection, exposure to study medication will not be applicable.

7.12 Efficacy Analysis

7.12.1 General consideration

In Part I, efficacy endpoints will be performed using the Safety Analysis Set. For efficacy endpoints, observations collected within 6 months of the last visit window will be included in analysis. Efficacy endpoints will be summarized on observed data by descriptive statistics only. No imputation will be performed.

In Part II, efficacy endpoints will be performed using the ITT Analysis Set. For efficacy endpoints, observations collected within 6 months of the last visit window will be included in analysis.

For efficacy analysis of study eyes in Part II, descriptive analyses will be provided. Additional analyses for handling missing data will be also provided for the primary endpoint and selected secondary endpoints. For the primary endpoint regarding the proportion of eyes with improved retinal sensitivity, the primary analysis to handle missing data is the last observation carried forward (LOCF) on locus level. Then the imputed value will be dichotomized for analysis based on the responder criteria. The supplementary analyses include the non-responder imputation (NRI) imputed on locus level, and multiple imputation on locus level followed by two-sample Binomial test.

For mean change from baseline in mean sensitivity, the primary analysis to handle missing data is multiple imputation on locus level followed by the mixed model repeated measure (MMRM) analysis. For selected secondary endpoints (BCVA and LLVA) regarding their change from baseline, the primary analysis of handling missing data is the MMRM analysis. For other continuous endpoints, summaries will be made by descriptive only.

For efficacy analysis of fellow eyes in Part II, only descriptive analysis will be provided.

7.12.2 MAIA Microperimetry

General considerations for Part I and Part II

When there are more than one microperimetry exam at the same visit, the latest assessment will be used for the purpose of the analysis.

MAIA microperimetry assessment is measured in decibel (dB) using a 10-2 grid of 68 points. Each point is labeled as '< 0', '0' or a positive integer (Ref. MAIA, Microperimetry Handbook). The point labeled as '< 0' is assigned a value of '-1' by MAIA in the calculation.

Improvement in Retinal Sensitivity in center grid is defined as an increase from baseline of 7 or more decibels in any 5 or more points out of the 16 central points.

Improvement in Retinal Sensitivity in whole grid is defined as an increase from baseline of 7 or more decibels in any 5 or more points of the grid as a whole (68 points).

The proportion of eyes with improved retinal sensitivity, for both the center grid and the whole grid, will be summarized by treatment groups and visit. They will be presented by study eye and fellow eye separately. The corresponding CI may be presented by using the Clopper-Pearson method.

Mean Sensitivity in center grid is defined as the mean in dB of the 16 points located in the center of the grid.

Mean Sensitivity in whole grid is defined as the mean in dB over the whole grid (all 68 points).

Mean Sensitivity, in both the center grid and the whole grid, including change from baseline will be summarized by treatment groups, visits and eyes. The corresponding CIs for actual values at each visit are calculated by one sample t-test, and for the change from baseline are calculated by paired t-test. The 3*IQR rule method is used to identify extreme outliers for the mean sensitivity change from baseline. If a subject whose mean sensitivity change from baseline from study eye or non-study eye at every post-baseline visit is > Q3+3*IQR or < Q1-3*IQR, where Q1 is the 1st quartile, Q3 is the 3rd quartile and IQR is the interquartile range, for mean change from baseline among all post-baseline visits, this subject is regarded to have anomalous mean sensitivity data and will be excluded from analysis along with clinical judgement.

For bivariate contour ellipse area 63%, bivariate contour ellipse area 95%, P1, and P2, their observed value and change from baseline will also be summarized by treatment groups and by visits. They will be presented by study eye and fellow eye separately. The corresponding CIs will be presented by one sample t-test.

Fixation stability is defined as: if P1≥75% then fixation stability is considered as stable. If P1< 75% and P2 ≥ 75% then fixation stability is considered as relatively unstable. Otherwise if P1< 75% and P2 <75%, then fixation stability is considered as unstable. If there are triplicates of P1 or P2 collected at a visit, the latest P1 or P2 record is selected for summary analysis. For fixation stability, shift from baseline will be summarized by shift from stable, relatively unstable, or unstable at baseline to stable, relatively unstable, or unstable at any post-baseline visit. The number and percentage of subjects within each category of fixation stability will be summarized by treatment groups and visits.

Part I

All analysis in Part I will be summarized by descriptive statistics.

The proportion of eyes with ≥ 7 dB improvement from baseline at ≥ 5 of 16 central loci, or ≥ 5 of 68 whole loci assessed by MAIA will be summarized by treatment groups, visits and eyes. The 95% CI will be calculated using the Clopper-Pearson method.

The secondary endpoint of change from baseline in mean sensitivity, in both the center 16 grid and the whole 68 grid assessed by MAIA microperimetry at 1, 2, 3, 6, 9, 12, 18 and 24 months will be summarized by treatment groups, visits and eyes. The 95% CI for mean will be calculated by one-sample t-test method, and for change from baseline will be calculated by paired t-test method.

Part II

The proportion of eyes with improved retinal sensitivity, for both the center 16 grid and the whole 68 grid will be summarized by treatment groups, visits and eyes.

Let p_{high}, p_{low}, and p_{untr} represent the proportions of eyes with ≥ 7 dB improved retinal sensitivity in 5 out of central 16 grid at Month 12 for the High Dose, Low Dose, and Untreated group, respectively.

The following 2 hypotheses will be tested:

- High Dose vs Untreated arm:

  H_{01}: $p_{high} = p_{untr}$ versus H_{11}: $p_{high} > p_{untr}$

- Low Dose vs Untreated arm:

  H_{01}: $p_{low} = p_{untr}$ versus H_{11}: $p_{low} > p_{untr}$

The proportion of eyes with improved retinal sensitivity, for both the center grid and the whole grid, at Month 12 will be compared between study arms (high dose vs untreated; low dose vs untreated) using the Fisher's Exact-Boschloo test with a Berger-Boos correction of beta=0.001 (Berger 1994). The difference in proportions between treatment groups will be presented with its corresponding 80% CI calculated using the method of Miettinen and Nurminen (Miettinen 1985).

- Primary Endpoint:

The intercurrent event is discontinuation of study early or lost to follow-up for this study. The estimand is the response rate difference between treatment and control group in ITT Analysis Set regardless of intercurrent events. The treatment policy strategy will be used for the intercurrent events. Observed data collected within 6 months outside the last visit window will be included in the analysis, including data collected after intercurrent events [ICH E9(R1) addendum 2019], i.e. discontinuation of study early or lost to follow-up.

For other missing data due to intercurrent events or other reasons, the following missing data imputation methods will be provided.

Primary analysis for missing data imputation:

The last observation carried forward method (LOCF) will be applied to the underlying continuous variables of retinal sensitivity. The imputed value will then be dichotomized based on the responder criteria, i.e., improvement of ≥7 dB from baseline. Imputation of retinal sensitivity will be done at locus level, i.e. if a locus value is missing at a visit, the last non-missing value will be carried over to this visit.

This is a hypothetical strategy based on the assumption that the retinal sensitivity of subjects who are discontinued from the study early or lost to follow up will remain unchanged from the last observed value until the month-12 visit. This is considered a plausible assumption due to 1) the slow progression of the disease for the untreated eyes, and 2) the durability of efficacy demonstrated for the treated eyes for up to 18 months based on the current Part I data.

In addition to the primary analysis, 2 supplementary analyses are proposed under different assumptions for the estimand to assess efficacy in subjects who have missing data.

Supplementary analysis for missing data imputation:

Supplementary Analysis I:

The composite strategy will be used for the intercurrent event. The non-responder imputation method will be applied on locus level. i.e. if a locus value is missing at a visit, it will be imputed as a failure. This is a conservative approach in that any missing data will be considered as a non-responder.

Supplementary Analysis II:

The hypothetical strategy will be used with the assumption that the retinal sensitivity of the subjects who have discontinued the study early or lost to follow-up will be similar to those subjects who remain in the study. The multiple imputation approach will be applied to the missing data on the locus level under the assumption of missing-at-random (MAR). MAR assumption is considered plausible since treatment is only administered once for gene therapy after randomization in the study. Subjects do not take ongoing doses after surgery. Subjects who are lost to follow up or drop out early are not related to the effect of ongoing dose taken. These missing data are regarded missing at random. The missing retinal sensitivity data at each locus will be imputed by the method of multiple imputation. Each imputed data set will be analyzed using two-sample Binomial test. The analyzed results from the imputed dataset will be combined based on Rubin's rule (Rubin 1987) for inference assuming the statistics estimated from each imputed dataset are normally distributed.

Sensitivity analysis

It was identified that microperimetry data from one site were impacted by a protocol deviation involving assessments conducted by an unmasked rather than masked assessor from month 3 to month 12. Microperimetry is an automated test that measures the minimum luminance of a light stimulus that can be perceived by a subject at defined locus of the retina. The impact of an unmasked assessor on results is limited due to the automated nature of the test, in that the assessor is not directly involved in generating or recording the results. In addition, the perceived light stimulus at a specific locus is tested by projecting multiple stimulus points of varying light intensities. The projection strategies further reduce the probability of an unmasked assessor significantly impact the data. However, an unmasked assessor can still introduce bias into the results in indirect ways, e.g., by encouraging the subject to stay focused during the test. The risk of significantly altering microperimetry results by an unmasked assessor is believed low.

Although the risk is low, to mitigate this risk a sensitivity analysis will be implemented by excluding mean sensitivity data assessed by unmasked assessor at Part II, Month 12. The sensitivity analysis will be applied to the summaries for the proportion of eyes with mean sensitivity ≥7 db improvement from baseline at ≥ 5 of the 16 loci at Month 12. The sensitivity analyses will be descriptive only and analyzed on observed case. For microperimetry data in Part II, the main analysis is still based on ITT analysis set. The

sensitivity analysis will be compared with the main analysis to assess the robustness of the data.

Exploratory analysis

- Secondary Endpoint:

The secondary endpoint is the change from baseline in mean sensitivity of the 16 central loci and 68 whole loci at 1, 2, 3, 6, 9 and 12 months. Observed data collected within 6 months outside the last visit window will be used regardless of intercurrent events, i.e. discontinuation of study early or lost to follow up. Observed value and change from baseline will be summarized by treatment groups, visits and eyes. The CI for observed value will be provided based on one-sample t-test, and the CI for change from baseline will be provided by paired t-test. The difference of change from baseline between treatment and control group at each visit will be provided. The CI and p-value for the difference will be also provided, which are calculated based on two sample t-test.

The primary analysis for handling missing data will be conducted by applying multiple imputation on each locus level, followed by the MMRM model under the assumption of MAR. Each imputed data set will use MMRM model to analyse change from baseline of the mean sensitivity, using fixed effects of treatment groups (high dose, low dose, and untreated), study visit, study visit-by-treatment interaction, baseline value of mean sensitivity and baseline-by-visit interaction. An unstructured covariance matrix will be used to model the

within-patient variance-covariance errors. If the unstructured covariance structure matrix results are in a lack of convergence, the heterogeneous Toeplitz covariance structure followed by the heterogeneous first-order autoregressive covariance structure will be used. The Kenward-Roger approximation will be used to estimate the denominator degree of freedom. Results from analysis of each imputed dataset, i.e., least square (LS) mean differences from placebo and their SEs, will be combined using Rubin's method [Rubin 1987] as implemented in the proc mianalyze procedure to produce a pooled LS mean estimate of treatment difference, and its CI, and a nominal pooled p-value.

To account for microperimetry data from the one site impacted by unmasked assessments from month 3 to month 12, a sensitivity analysis will be implemented for central 16-loci mean sensitivity of observed value and change from baseline at Month 12, by excluding mean sensitivity data points assessed in an unmasked fashion at Month 12. The sensitivity analysis will be descriptive only and analyzed on observed case. The sensitivity analyses will be compared with analysis made by the ITT analysis set to assess the robustness of the data.

7.12.3 Best-Corrected Visual Acuity (BCVA)

Where there are more than one BCVA measurement at the same visit, the median value will be used for the purpose of the analysis.

Part I

BCVA actual values and change from baseline at 1, 3, 6, 9, 12, 18, and 24 months will be summarized by treatment groups, visits, and eyes based on observed case. The 95% CI for mean values and mean change from baseline will be provided.

Furthermore, the proportion of eyes with BCVA gain from baseline ≥ 5, 10, 15 letters will be summarized by treatment groups, visits and eyes. The proportion of eyes with BCVA change from baseline > -5 letters measuring the maintenance of BCVA will also be summarized by treatment groups, visits, and eyes. The 95% CI will be provided by using the Clopper-Pearson method.

Part II

Change from baseline in BCVA at 1, 2, 3, 6, 9, and 12 months will be summarized by descriptive statistics. The 80% CI for mean values and mean change from baseline will be provided. Observed data collected within 6 months of the last visit window will be used regardless of intercurrent events, i.e. discontinuation of study early or lost to follow up.

For handling the missing data imputation for analyzing the change from baseline, the primary analysis is:

MMRM model will be applied to analyse the mean change from baseline, using fixed effects of treatment groups (high dose, low dose, and untreated), study visit, study visit-by-treatment interaction, baseline BCVA value and baseline-by-visit interaction. An unstructured covariance matrix will be used to model the within-patient variance-covariance errors. If the unstructured covariance structure matrix results are in a lack of convergence, the heterogeneous Toeplitz covariance structure followed by the heterogeneous first-order autoregressive covariance structure will be used. The Kenward-Roger approximation will be used to estimate the denominator degree of freedom. MMRM assumes missing data to be missing at random.

The proportion of eyes with BCVA gain from baseline ≥ 5, 10, 15 letters, as well as the BCVA change from baseline >-5 (maintenance) will be summarized based on observed case

within each group and the 80% CI for proportion will be calculated by the Clopper-Pearson method. For BCVA gain from baseline ≥ 10 and ≥ 15 letters, where subjects are expected to show more pronounced improvement in treated eyes than non-treated eyes, the difference in proportion between the treatment group and control group will be presented with its corresponding 80% CI calculated using the method of Miettinen and Nurminen (Ref. Miettinen and Nurminen, 1985), and the p-value will be reported by the Fisher's Exact-Boschloo test with a Berger-Boos correction of beta=0.001(Berger 1994). In the event of missing BCVA data, the primary analysis to analyse the proportion of study eyes with BCVA gain or maitenenace from baseline is LOCF approach.

To account for BCVA data from one site impacted by a protocol deviation involving assessments conducted by an unmasked rather than masked assessor from month 3 to month 12, a sensitivity analysis will be conducted evaluating safety signals for BCVA loss from baseline. A summary table of BCVA loss from baseline ≥ 5, ≥ 10, ≥ 15 letters will be summarized at each visit, by excluding BCVA data impacted by unmasking.

7.12.4 Low Luminance Visual Acuity (LLVA)

When LLVA are measured in triplicates, the median value will be used for analysis.

Part I

LLVA actual values and change from baseline will be summarized by treatment groups, visit, and eyes. The 95% CI for mean values and mean change from baseline will be provided. The proportion of eyes with LLVA gain from baseline ≥ 5, 10, 15 letters will be summarized, as well as the proportion of eyes with LLVA change from baseline > -5 letters will be summarized. The 95% CI will be provided by using the Clopper-Pearson method.

Low luminance deficit (LLD) is the difference between BCVA and LLVA. LLD observed values and change from baseline will be summarized by treatment groups, visits, and eyes.

Part II

The analysis conducted for BCVA will be applied to LLVA. The primary analysis for handling missing data for change from baseline is using MMRM model.

The proportion of eyes with LLVA gain from baseline ≥ 5, 10, 15 letters, as well as the LLVA change from baseline >-5 letters (maintenance) will be summarized within each group and the 80% CI for proportion will be provided. For LLVA gain from baseline ≥ 10 and ≥ 15 letters, where subjects are expected to show a more pronounced improvement in treated eyes than non-treated eyes, the difference in proportions between treatment group and control group will be presented with its corresponding 80% CI calculated by Miettinen and Nurminen, and the p-value will be reported by the Fisher's Exact-Boschloo test with a Berger-Boos correction of beta=0.001.In the event of missing LLVA, the primary analysis of the proportion of study eyes with LLVA gain or maintenance from baseline is the LOCF approach.

LLVA data from one site were impacted by a protocol deviation involving assessments conducted by an unmasked rather than masked assessor from month 3 to month 12. LLVA improvement from baseline is regarded as an efficacy endpoint. To futher detect the impact of unmasking on this efficacy signal, a sensitivity analyses will be conducted to summarize the proportion of eyes with LLVA change from baseline ≥ 5, ≥ 10, and ≥15 letters, as well as change from baseline >-5 (maintenance) at Month 12, by excluding LLVA data impacted by unmasking at Month 12.

7.12.5 Spectral Domain Optical Coherence Tomography (SD-OCT)

Part I and Part II

For foveal subfield thickness, central horizontal ellipsoid width (μm), central ellipsoid area (mm^2), choroidal thickness at foveal center, and total macular volume change from baseline will be summarized by treatment groups, visits and eyes based on observed case.

For presence of vitreomacular traction (VMT), epiretinal membrane (ERM), macular hole in vitreo-macular interface disease, cystoid macular edema, subretinal fluid, and targeted ocular findings (TOF) will be summarized by treatment groups, visits, and eyes.

The shift table for VMT, ERM and macular hole will be summarized from "absent", or "present" at baseline to "absent" or "present" at any post-baseline visit.

The shift table for cystoid macular edema and subretinal fluid will be summarized from "none", "Yes, foveal" or "Yes, non-foveal" at baseline to "none", "Yes, foveal", or "Yes, non-foveal" at any post baseline visit.

7.12.6 Fundus Autofluorescence

Part I and Part II

For total area of preserved autofluorescence and distance from foveal center (FC) to nearest border of preserved autofluorescence, their observed value and change from baseline will be summarized by treatment groups, visits and eyes based on observed case.

The number and percentage of subjects with subfoveal or non-subfoveal location of lesion in relation to foveal center, and presence of targeted ocular findings (TOF) will be summarized by treatment groups, visits, and eyes.

The shift from baseline in the location of lesion in relation to autofluorescence will be summarized from "subfoveal", or "non-subfoveal" at baseline to "subfoveal", or "non-subfoveal" at any post baseline visit.

7.12.7 Visual Fields

Part I and Part II

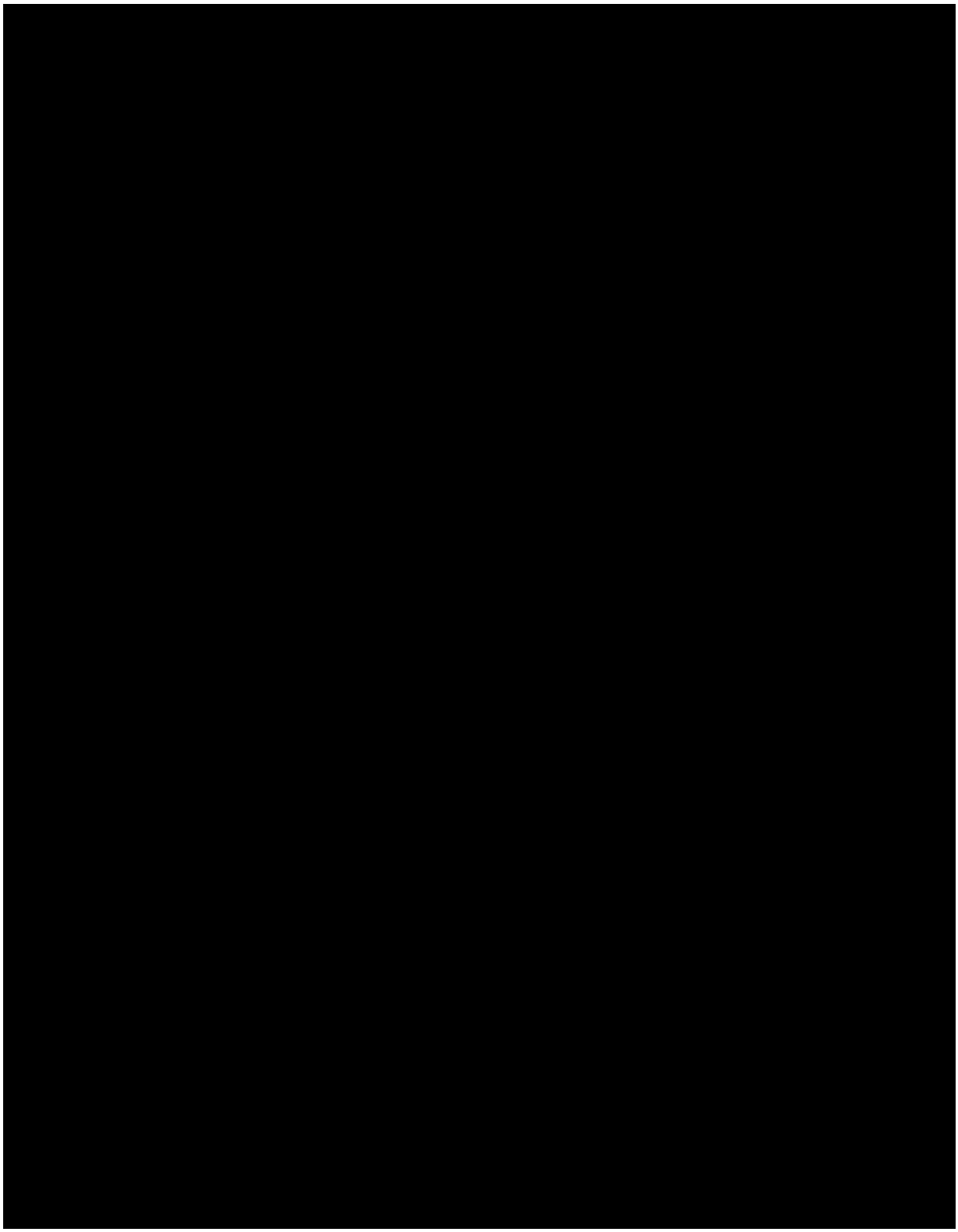
When there is more than one visual field measurement at the same visit, the average will be used for the purpose of the analysis.

The Reliability Factor (RF) is equal to (number of false positive responses + number of false negative responses) divided by (number of false positive presentations + number of false negative presentations) times 100. If there are 0 responses, then the RF value is equal to 0. RFpositive is defined as (number of false positive responses) divided by (number of false positive presentations) times 100.

If RF$\leq$ 20% the measurement is considered reliable. If 20% < RF $\leq$ 25% and RFpositive $\leq$ 10% the measurement is also considered reliable. Otherwise if 20% < RF $\leq$ 25% and RFpositive > 10%, or RF > 25%, the measurement is not reliable.

For reliable measurement from the volume of full field hill of vision and volume of 30-degree hill of vision, their observed values and change from baseline will be summarized by treatment groups, visits and eyes on observed case. In addition, summary tables from full records of full field hill of vision and volume of 30-degree hill of vision will be also

provided. A listing including all records, as well as an indication of reliable records will be provided.



7.12.10 Contrast Sensitivity

Part I and Part II

Contrast Sensitivity Score (CSS) and change from baseline will be summarized by treatment groups, visits and eyes by observed case.

7.13 Safety Analysis

Safety analysis will be performed using the Safety Analysis Set. All safety analyses will be summarized based on observed case.

7.13.1 Adverse Events

Part I and Part II

Treatment Emergent Adverse Events (TEAEs) are defined as AEs starting on or after the day of the surgery. Summary tables will include TEAEs only. TEAEs will be summarized by ocular events (study eye vs. fellow eye), non-ocular events within each treatment group. Non-treatment emergent AEs will be included in the listing.

The number and percentage of subjects reporting TEAE event, non-ocular event, ocular event, any ocular event in the study eye and in the fellow eye will be summarized.

Both counts of subjects and events will be reported. A subject with more than one occurrence of the same AE in a particular system organ class will be counted only once in the total of those experiencing AEs in that particular system organ class. If a subject has multiple

events under the same category of severity, relationship, outcome, or action taken, they are counted only once in each category.

When a plausible relationship is identified by the Investigator, it will be reported in the CRF as related to study drug, related to study procedure, related to both study drug and study procedure, or unknown. In the summary tables, related to study drug events are defined as events assessed by the investigator to be related to study drug or related to both study drug and study procedure. Related to study procedure events are defined as events assessed by the investigators to be related to study procedure or related to both study drug and study procedure.

An ocular event is an event where the site of the event is reported as OU, OD or OS. Where an event is recorded as 'OU', it will be counted once for each eye (study eye and fellow eye).

Time to onset will be calculated as the day of onset minus the day of treatment (surgery). The categories for time-to-onset for ocular inflammation and vision acuity reduced are defined as time to onset ≤ 30 days and > 30 days. The list of preferred terms assigned to ocular inflammation and visual acuity loss are based on custom search strategy and are provided in a separated document. The following analyses will be provided:

- Ocular Inflammation-related TEAEs by SOC and PT
- Time to onset of first occurrence of Ocular Inflammation-related TEAEs by SOC and PT, and by time to onset (≤30 days; >30 days)
- Time to onset of first occurrence of Ocular Inflammation-related TEAEs by SOC and PT, and by time to onset (≤90 days; >90 days)
- Visual Acuity Reduced-related TEAEs by SOC and PT
- Time to onset of first occurrence of Visual Acuity Reduced-related TEAEs by SOC and PT, and by time to onset (≤30 days; >30 days)
- Time to onset of first occurrence of Visual Acuity Reduced-related TEAEs by SOC and PT, and by time to onset (≤90 days; >90 days)

For example, if a subject has multiple TEAE events on the same preferred term level, the first occurrence within each latency period is counted. i.e. if a subject has multiples TEAEs on the same preferred term level where some start within ≤ 30 days and others start > 30 days post treatment, the first TEAE record within ≤ 30 days is counted. Similarly the first TEAE record > 30 days is counted.

AEs will be coded using MedDRA version 23.1.

In addition, TEAE will be summarized in the following tables:

- Overall summary of TEAE
- TEAEs by SOC and PT sorted by decreasing frequency
- Dose Limiting Toxicity (DLT) AEs, by SOC and PT
- TEAEs with an incidence of 10% or more in any treatment group, by SOC and PT
- Study drug-related only TEAEs by SOC and PT
- Study procedure-related only TEAEs by SOC and PT
- Study drug and study procedure related TEAEs, by SOC and PT

- Related TEAEs but with unknown attribution to study drug or study procedure, by SOC and PT
- SAEs by SOC and PT sorted by decreasing frequency
- Study drug-related SAEs, by SOC and PT (only study drug-related)
- Study procedure-related SAEs, by SOC and PT (only study procedure-related)
- Study drug and study procedure related SAEs, by SOC and PT
- Related SAEs but with unknown attribution to study drug or study procedure, by SOC and PT
- TEAEs by maximum severity, by SOC and PT

The following AE listings will be provided:

- Ocular adverse event – treatment emergent
- Ocular adverse event – non-treatment emergent
- Non-ocular adverse event – treatment emergent
- Non-ocular adverse event – non-treatment emergent
- Serious adverse events (SAEs)
- SAEs leading to discontinuation from the study
- A listing of death

7.13.2 Best-Corrected Visual Acuity (BCVA)

Part I and Part II

The proportion of eyes with a ≥5 letters, a ≥10 letters and a ≥15 letters decrease from baseline will be summarized by treatment groups, visits, and eyes.

When there are more than one BCVA measurement at the same visit, the median value will be used for the purpose of the analysis.

7.13.3 Low Luminance Visual Acuity (LLVA)

Part I and Part II

The proportion of eyes with a ≥5 letters, a ≥10 letters and a ≥15 letters decrease from baseline will be summarized by treatment groups, visits, and eyes.

When there are more than one LLVA measurement at the same visit, the median value will be used for the purpose of the analysis.

7.13.4 Dilated Ophthalmoscopy

Part I and Part II

The number and percentage of subjects within each category of each Dilated Ophthalmoscopy outcomes will be summarized by treatment groups, visits and eyes. Shift from baseline will also be evaluated.

The shift for “Vitreous”, “Macula”, “Peripheral retina”, “Choroid” and “Optic nerve” will be summarized from “Normal/Clinically insignificant abnormality”, or “Clinically significant abnormality” at baseline to “Normal/Clinically insignificant abnormality”, or “Clinically significant abnormality” at any visit post-baseline.

The shift for “Retinal tear(s)” and “Retinal detachment” will be summarized from “Absent”, or “Present” at baseline to “Absent”, or “Present” at any visit post-baseline.

7.13.5 Slit Lamp Examination

Part I and Part II

The number and percentage of subjects within each category of slit lamp examinations outcomes will be summarized by treatment groups, visits and eyes.

The shift for ‘Cornea’, ‘Conjunctiva’, ‘Iris’, ‘Lens’ and ‘Anterior Segment’ will be summarized from “Normal/Clinically insignificant abnormality” or “Clinically significant abnormality” at baseline to “Normal/Clinically insignificant abnormality”, or “Clinically significant abnormality” at any post-baseline visit.

The categories for the shift from baseline for ‘Anterior chamber, hypopyon’, ‘Grading of anterior chamber cells’, ‘Grading of anterior chamber flare’ and ‘Vitreous inflammation quantification’ assessments are defined as follow:

- Absent at Baseline to absent
- Absent at Baseline to present
- Present at Baseline to absent
- Present at Baseline to present

For ‘Grading of anterior chamber cells’ assessment, absent is defined as ‘0 Cells’ and present is defined as any other category (except missing).

For ‘Grading of anterior chamber flare’ assessment, absent is defined as ‘Complete absence’ and present is defined as any other category (except missing).

For ‘Vitreous inflammation quantification’ assessment, absent is defined as ‘0’ and present is defined as any other category (except missing).

7.13.6 Intraocular Pressure (IOP)

Part I and Part II

Intraocular pressure and change from baseline will be summarized by treatment groups, visits, and eyes.

7.13.7 Lens Opacity Grades

Part I and Part II

The number and percentage of subjects within each category of nuclear opalescence grade, nuclear color grade, cortical cataract grade and posterior cataract grade will be summarized by treatment groups, visits, and eyes.

The categories for lens opacity grades are defined as category 1, 2, 3 and 4. Category 1 includes values 1, 1.0 and 0.x. Category 2 includes values 2, 2.0 and 1.x. Category 3 includes values 3, 3.0 and 2.x. Category 4 includes values 4, 4.0 and 3.x.

The shift of lens opacity grade will the summarized from “1”, “2”, “3”, “4” at baseline to “1”, “2”, “3”, “4” at any post-baseline visit.

7.13.8 Fundus Photography

Part I and Part II

Presence and severities (none, mild, moderate, severe) of retinal pigment epithelium (RPE) hyperplasia, retinal arteriolar narrowing, retinal vessel sheathing, optic atrophy/pallor and optic disc swelling and presence of targeted ocular findings (TOF) will be analyzed.

The shift of fundus photograph will be summarized from “none”, “mild”, “moderate”, “severe” at baseline to “none”, “mild”, “moderate”, “severe” at any post baseline visit.

7.13.9 Vital Signs

Part I and Part II

For systolic blood pressure, diastolic blood pressure and heart rate, their observed values and change from baseline will be summarized by treatment groups and visits.

7.13.10 Laboratory Assessments

7.13.10.1 Safety Assessments

Part I and Part II

For the purposes of summarization in both the tables and listings, all laboratory values will be converted to standardized units. Laboratory abnormalities will be graded using the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. For numeric laboratory parameters the observed lab values and mean change from baseline will be summarized at each visit. Clinically significant laboratory data will be summarized using shift tables where appropriate. For each subject, the lab values in hematology and blood chemistry will be flagged as “low”, “normal”, or “high” relative to the normal ranges of the central laboratory, or as “unknown” if no result is available.

For blood chemistry and serum chemistry, shifts tables from baseline to low/high post-baseline will be presented. Shift to low includes normal to low, high to low, and unknown to low. Shift to high includes normal to high, low to high, and unknown to high. For a subject if a lab is collected multiple times at post baseline, as long as one lab value is low this subject is regarded to have “low” post-baseline for this lab parameter. Similarly, if at least one post-baseline lab value is “high” this subject is regarded to have “high” post-baseline for this lab parameter.

Markedly abnormal indicate clinically significant laboratory abnormalities determined by Biogen. For hematology and blood chemistry the number and percentage of subjects with markedly abnormal laboratory post-baseline will be summarized by treatment group in Part I and Part II following the criteria in Table 2. Shifts tables from baseline to markedly abnormal post-baseline will also be summarized. For a subject if a lab is collected multiple times at post baseline, as long as one lab value is markedly abnormal this subject is counted to be markedly abnormal for this lab parameter.

Listings of subject’s laboratory data for each visit will be presented.

Table 1: Criteria for markedly abnormal laboratory

Parameter name	Unit	Low	High
Hematology			
White blood cells	$x10^9$ cells/L	<3.0	>16
Lymphocytes	$x10^9$ cells/L	<0.8	>12
Neutrophils	$x10^9$ cells/L	<1.5	>13.5
Monocytes	$x10^9$ cells/L	N/A	>2.5
Eosinophils	$x10^9$ cells/L	N/A	>1.6
Basophils	$x10^9$ cells/L	N/A	>1.6
Red blood cells (RBC)	$x10^{12}$ cells/L	≤ 3.5	≥ 6.4
Hemoglobin - Females	g/L	≤ 95	≥ 175
- Males		≤ 115	≥ 190
Hematocrit - Females	%	≤ 32	≥ 54
- Males		≤ 37	≥ 60
Platelet count	$x10^9$ cells/L	≤ 75	≥ 700
Blood Chemistry			
Alanine aminotransferase (ALT)		N/A	> 3 x ULN
Aspartate aminotransferase (AST)		N/A	> 3 x ULN
Alkaline phosphatase (ALP)		N/A	>3 x ULN
Total bilirubin		N/A	>2 x ULN
Blood urea nitrogen (BUN)		N/A	≥10.7 mmol/L
Creatinine		N/A	≥176.8 umol/L
Sodium	mmol/L	≤ 126	≥ 156
Potassium	mmol/L	≤ 3	≥ 6
Chloride	mmol/L	≤ 90	≥ 118
Bicarbonate	mmol/L	≤ 16	≥ 35

7.13.10.2 Viral Shedding

If a suitable sample is developed and validated upon data base lock, the following analyses will be performed.

Presence or absence of AAV8 DNA in different bodily fluids (tears [each eye], saliva, blood, and urine) will be determined by quantitative polymerase chain reaction (qPCR) at screening and subsequent visits. For each sample type, the number and percentage of positive, negative, and below level of quantification (BLQ) samples will be summarized by treatment groups and visits. In addition, listings of individual viral shedding results will be provided.

7.13.10.3 Immunogenicity

The immunogenicity analyses will be based on subjects who have at least one post-surgery sample evaluable for immunogenicity from safety analysis set.

Immunogenicity encompasses neutralizing antibody (NAb) assay, enzyme-linked immunospot (ELISpot) assay and anti-drug antibody (ADA) assay.

NAb

If a suitable NAb assay is developed and validated upon data base lock, the following analyses will be performed.

Serum NAb levels at screening and subsequent visits will be reported as the reciprocal of the highest fold of dilution (titer) needed to rid the sample of neutralizing activity against an AAV8 reporter virus. The results will be reported as "<1", ">15,000", and continuous values between 1 and 15,000. Results of "<1" are regarded as negative, and results of ">1" are regarded as positive.

The baseline value for NAb is defined as the last available value prior to surgery of the treated eye. For subjects without any assessments prior to surgery of the treated eye, Day 1 sample will be used as baseline. If both prior to surgery and Day 1 assessments are not available, NAb samples at baseline will be regarded as missing.

Pre-existing immunoreactivity is defined as a positive NAb assay response at baseline. Treatment-emergent positive is defined as any post-treatment positive NAb assay response when the baseline NAb result is negative. Treatment-boosted is defined as any post-treatment positive NAb assay response greater than or equal to a 4-fold change over baseline titer level, when baseline NAb assay is positive.

The number and percentage of subjects with positive and negative NAb assay will be summarized by treatment groups and visits. The number and percentage of subjects with pre-existing immunoreactivity, treatment emergent positive, treatment-boosted, and negative at both screening and post-baseline will be also summarized by treatment groups. Listing of individual NAb result will be provided.

To determine the relationship between NAb and post-treatment ocular inflammation related adverse events, the NAb status (pre-existing immunoreactivity, treatment emergent positive, treatment-boosted, and negative at both screening and post-baseline) will be summarized for subjects who experience ocular inflammation adverse events post treatment. The ocular inflammation adverse event is defined from the custom search strategy. The impact of NAb status on other safety/efficacy may be further evaluated when sufficient data are obtained.

ELISpot

If a suitable ELISpot assay is developed and validated upon data base lock, the following analyses will be performed.

Cellular immunity mediated by cytotoxic T lymphocytes (CTL) will be evaluated by Interferon-gamma (IFN- γ) ELISpot assay of peripheral blood mononuclear cells (PBMC). Cellular immunity will be evaluated at baseline and post-baseline visits according to the protocol's schedule of events and is reported positive or negative by peptide pool stimulation and as spot forming units (SFU) per 10^6 PBMC.

The number of subjects and percentage with positive and negative ELISpot will be summarized by treatment groups and visits. A listing of individual ELISpot data will be provided. The potential relationship between ELISpot and safety/efficacy may be further evaluated when sufficient data are available.

Anti-Drug Antibody (ADA)

An assay for measuring anti-RPGR protein antibody is under development. If a suitable assay is developed and validated upon data base lock, the following analyses will be performed.

The baseline value for ADA is defined as the last available value prior to surgery of the treated eye. For subjects without any assessments prior to surgery of the treated eye, Day 1 sample will be used as baseline. If both prior to surgery and Day 1 assessments are not available, ADA samples at baseline will be regarded as missing.

Pre-existing immunoreactivity is defined as a positive ADA assay response at baseline. Treatment-emergent positive is defined as any post-treatment positive ADA assay response when the baseline ADA assay is negative. Treatment-boosted is defined as any post-treatment positive ADA assay response that is greater than or equal to a 4-fold change over baseline titer level, when baseline ADA assay is positive.

The number and percentage of subjects with positive or negative ADA assay at each visit will be summarized. The number and percentage of subjects with pre-existing immunoreactivity, treatment-emergent positive, treatment-boosted, and negative at both screening and post-baseline will be summarized by treatment groups. Listings of ADA positivity/negative status and titers will be presented by subject and time point.

To determine the relationship between ADA and post-treatment ocular inflammation related adverse events, the ADA status (pre-existing immunoreactivity, treatment emergent positive, treatment-boosted, and negative at both screening and post-baseline) maybe evaluated for subjects who experience ocular inflammation adverse events when sufficient data are available. The impact of ADA assay on other safety/efficacy may be further evaluated when sufficient data are obtained.

7.14 Protocol Deviations

Protocol deviations identified during site monitoring will be captured in a protocol deviation log and categorized as major or minor deviations. The major protocol deviations and COVID-19-Related protocol deviation maybe be summarized by treatment groups. Listings will be generated for the major and minor protocol deviations.

At Dr. [redacted] ([redacted]), it was identified that microperimetry data and some non-microperimetry data were assessed by unmasked assessors at the scheduled visits from Month 3 to Month 12, a finding that is noncompliant with the masking procedure specified in protocol. The proportion of eyes with mean sensitivity ≥7 db improvement from baseline at ≥ 5 of the 16 loci at Month 12 is the primary endpoint, and month 12 is the visit for the primary analysis. The mean sensitivity data collected by unmasked assessor at Month 12 are regarded as major protocol deviations. Data impacted by other visits are regarded as minor protocol deviations. Other endpoints including the secondary endpoint of BCVA, and [redacted], were also assessed by unmasked instead of masked assessors at the scheduled visits from Month 3 to Month 12. The impacts on the study read-out from the secondary [redacted] endpoints are deemed small and they are minor protocol deviations. The handling of these impacted data is described in sections 7.12.2, 7.12.3, and 7.12.4.

7.15 Deviations from SAP

Any deviations from the original statistical plan will be described and justified in the final clinical study report.

7.16 Selected Algorithms/SAS Codes

- **Descriptive statistics for continuous variables:**

```
PROC UNIVARIATE DATA=dset NOPRINT;
VAR var1 var2 var3 …varn;
BY byvar; (optional)
OUTPUT OUT=outname
N=n MEAN=mean MIN=min MAX=max MEDIAN=median STD=std
P5=p5 P95=p95 Q1=q1 Q3=q3;
RUN;
```

- **80% CIs for mean of observed (actual) value– one sample t-test:**

```
proc ttest data=sample alpha=0.20;
class treatment;
var endpoint;
run;
```

- **80% CIs for mean change from baseline– paired ttest formula:**

```
proc ttest data=dset alpha = 0.20;
paired var1*var2;
run;
```

- **Clopper-Pearson 80% CIs within group for binomial proportions:**

```
data one;
input group response count;
datalines;
1 1 61
1 2 39
2 1 25
2 2 15
3 1 0
3 2 5
;
run;

proc freq data=one;
tables response/binomial (exact) alpha=0.20 missprint;
weight count;
run;
```

- **80% CI for difference in proportion by the method of Miettinen and Nurminen**

```
Proc freq data=dataset noprint;
tables group*outname/riskdiff(cl=mn) measure alpha = 0.20;
Output out = outname;
Run;
```

- **Mixed Model Repeated Measures (MMRM)**

```
Proc mixed DATA = data;
CLASS subjid tr01pg1 visit;
MODEL chg = tr01pg1 bs visit visit*tr01pg1 bs*visit / DDFM=KENWARDROGER Solution;
REPEATED visit / TYPE=UN SUBJECT=subjid;
LSMEANS visit*tr01pg1/cl pdiff;
Run;
```

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