External Natural History Controlled, Open-Label Intervention Study to Assess the Efficacy and Safety of Long-Term Treatment with Raxone® in Leber's Hereditary Optic Neuropathy (LHON)

Study IDs: SNT-IV-005 (LEROS)

STATISTICAL ANALYSIS PLAN

Version 1.0

07 June 2021

Santhera Pharmaceuticals (Switzerland) Ltd.

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1 Abbreviations

2nd eye onset	Most Recent Affected Eye at Baseline
AE(s)	Adverse Event(s)
AESI	Adverse Event of special interest
BC	Blindness Category
BL	Baseline
CI	Confidence Interval
CRB	Clinically Relevant Benefit
CRR	Clinically Relevant Recovery
CRS	Clinically Relevant Stabilization
CRS-1	Case Record Survey SNT-IR-006
CRS-2	Case Record Survey SNT-CRS-002
CRW	Clinically Relevant Worsening
CS	Clinically Significant
CSP	Clinical Study Protocol
CSR	Clinical Study Report
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
IDE	Idebenone
ITT	Intent-To-Treat
KM	Kaplan-Meier
LEROS	Open-label study SNT-IV-005
LHON	Leber's Hereditary Optic Neuropathy
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intent-to-Treat
NCS	Not Clinically Significant
NH	Natural History
OLS	Open-label study
PT	Preferred Term
RKM	Reverse Kaplan-Meier
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation

SE	Standard Error
SOC	System Organ Class (SOC)
TEAE	Treatment Emergent Adverse Events
VA	Visual Acuity

2 Introduction

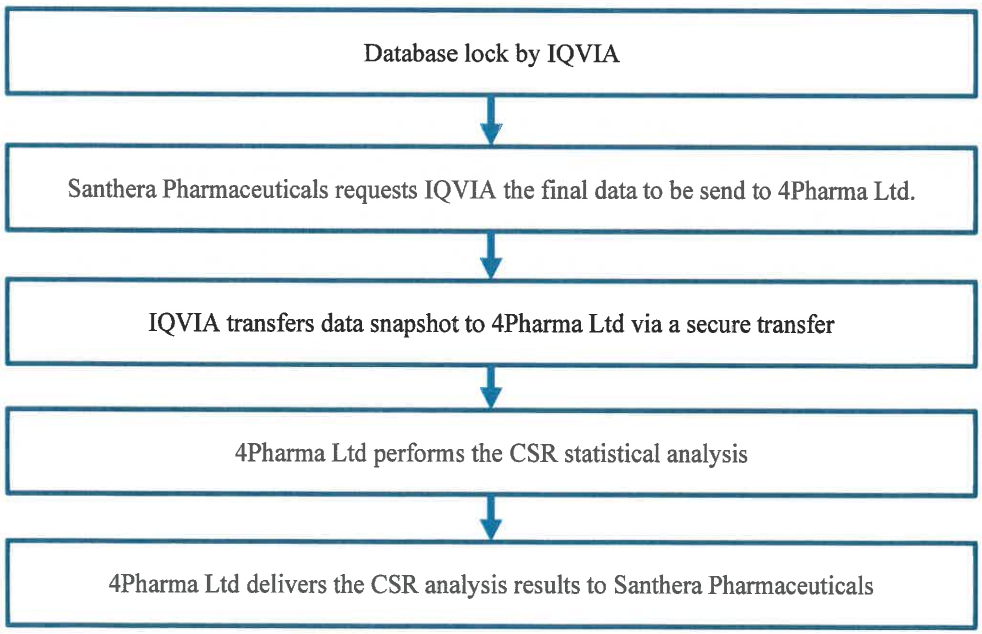
This Statistical Analysis Plan (SAP) defines the analysis of data from the open-label interventional study LEROS (SNT-IV-005), describing the disease progression in LHON patients treated with Raxone® (idebenone), compared with the two case-report survey studies CRS-1 (SNT-IR-006) and CRS-2 (SNT-CRS-002) providing the external natural history control group of idebenone naïve patients. The SAP will be finalized before database lock of LEROS.

EDC system maintenance, data management, and monitoring at the trial site is performed by IQVIA (previously Quintiles). Medical Monitoring is performed by a Consultant to and Santhera Pharmaceuticals (Switzerland) Ltd. Data Analysis is performed by 4Pharma Ltd. Santhera Pharmaceuticals (Switzerland) Ltd will not have access to the data snapshot before database lock. Figure 1 shows the steps which will occur since database lock until final CSR data analysis results.

This SAP provides details on the conduct of the data analysis described in SNT-IR-006 Clinical Study Protocol (CSP) version 1 dated 17 May 2013, SNT-CRS-002 CSP version 1.0 dated 5 April 2016, SNT-IV-005 CSP version 3.0 dated 06 March 2019, SNT-IV-005 CSP version 2.1_US and SNT-IV-005 CSP addendum COVID-19 version1.0 dated 29 May 2020; and specifies additional statistical analyses that will be performed.

This SAP takes precedence over the current protocols in any case of discrepancies.

Figure 2 – Database lock data transfer process



3 Objectives

3.1 Primary Objective

To assess efficacy of Raxone® in the promotion of recovery or stabilization of Visual Acuity (VA) in LHON patients treated with Raxone® ≤1 year after the onset of symptoms, compared to an external Natural History (NH) control group of idebenone (IDE) naïve patients

3.2 Secondary Objectives

- To assess efficacy of Raxone® in the promotion of recovery or stabilization of VA in LHON patients treated with Raxone® >1 year after the onset of symptoms, compared to an external NH control group of IDE naïve patients
- To compare the promotion of recovery or stabilization of VA in LHON patients treated with Raxone® ≤1 and >1 year after the onset of symptoms
- To assess the influence of mutation on the promotion of recovery or stabilization of VA in LHON patients treated with Raxone®
- To assess the influence of time since onset of symptoms prior to the initiation of treatment with Raxone® on the promotion of recovery or stabilization of VA in LHON patients
- To assess the influence of duration of treatment with Raxone® on changes in VA in LHON patients
- To assess safety of long-term treatment of LHON patients with Raxone®

Raxone has been granted an EU marketing authorization under exceptional circumstances. Accordingly, the Marketing Authorization Holder is conducting a program of studies as post-authorization measures. One of the specific obligations of this program is the conduct of the LEROS study. The study objectives were reviewed and agreed upon during the centralised procedure as part of the Risk Management Plan (RMP) assessment.

4 Endpoints

4.1 Primary Endpoint

- Proportion of eyes with clinically relevant recovery of visual acuity from Baseline (BL) or in which BL VA better than 1.0 logMAR was maintained at Month 12 in patients treated with Raxone® ≤1 year after the onset of symptoms, compared to matching external natural history control group

Clinically Relevant Recovery (CRR) is defined as a change from "off-chart" VA to at least 1.6 logMAR value or an improvement of at least 0.2 logMAR value within "on-chart".

4.2 Secondary Endpoints

- Components of the primary endpoint:
    - Proportion of eyes with CRR of VA from BL at Month 12 compared to matching external NH control group
    - Proportion of eyes in which BL VA better than 1.0 logMAR was maintained at Month 12 compared to matching external NH control group
- Proportion of eyes in patients treated with Raxone® >1 year after the onset of symptoms with CRR of VA from BL or in which BL VA better than 1.0 logMAR was maintained at Month 12 compared to external NH control group, in all patients and classified by mutation
- Proportion of eyes and patients treated with Raxone® ≤1 year after the onset of symptoms with CRR of VA from BL or in which BL VA better than 1.0 logMAR was maintained following 6, 18 and 24 months of treatment with Raxone® compared to matching external NH control group, in all patients and classified by mutation
- Proportion of eyes/patients treated with Raxone® ≤1 year or >1 year after the onset of symptoms with "Off-chart" VA at BL in whom VA improves to better than 1.60 logMAR by Month 6, 12, 18 and 24
- Proportion of eyes/patients treated with Raxone® ≤1 year or >1 year after the onset of symptoms with VA in the categories of better than 1.0 logMAR, 1.0 to 1.68 logMAR and above 1.68 logMAR at each assessment time point up to Month 24
- Safety as assessed by AE count and, as available, laboratory analyses during the study.

5 Design and type of the study

The Sponsor has been granted an EU marketing authorisation (MA) under exceptional circumstances for Raxone® (idebenone 150 mg) for the treatment of visual impairment in adolescent and adult patients with Leber's Hereditary Optic Neuropathy (LHON) in September 2015.

Pursuant to Article 14(8) of Regulation (EC) No 726/2004, the Marketing Authorisation Holder (MAH) is conducting a program of studies as post-authorisation measures (PAMs) to further investigate the benefits and safety of Raxone® in the treatment of LHON patients.

One specific obligation consists of the LEROS study (SNT-IV-005): an open-label interventional Phase IV study, designed to further assess the efficacy and safety of Raxone® in the long-term treatment of LHON patients. According to European legislation and regulatory guidelines, LEROS qualifies as a Post Approval Efficacy Study (PAES).

To assess efficacy of Raxone® in the promotion of recovery or stabilization of visual acuity in patients treated with Raxone data is compared to an external natural history control group of idebenone naïve patients (CRS-1 and CRS-2).

LEROS is a multi-country, multi-centre, open-label interventional study with 80 patients to be enrolled ≤1 year after the onset of symptoms to complete the 12 months treatment period (for evaluation of the primary endpoint), and 80 patients enrolled >1 year after the onset of symptoms to complete the 12 months treatment period.

Detailed study procedures and design is described in the described in the LEROS CSP.

CRS-1 is a multi-country, multi-centre, historical case record survey which collected historically documented VA data for all patients with a genetically confirmed diagnosis of LHON. No exclusion criteria were applied. Detailed study procedures and design is described in the CRS-1 CSP.

CRS-2 is a multi-country, multi-centre, historical case record survey which collected data on the VA of eligible patients from existing medical records. Detailed study procedures and design is described in the CRS-2 CSP.

5.1 Case Record Survey SNT-IR-006 inclusion criteria

The survey will collect historically documented visual acuity data for all patients at participating sites with a genetically confirmed diagnosis of LHON. No exclusion criteria apply. Patients are not required to attend the clinic for the survey.

5.2 Case Record Survey SNT-CRS-002 inclusion criteria

Case Records from patients will be collected from ALL patients of the participating sites who fulfil the following prospectively defined inclusion criteria:

- Age ≥ 12 years
- The onset of symptoms is dated after 1999 and is well documented (at least month of onset of symptoms is known for each eye)
- At least two VA assessments are available within 5 years of onset of symptoms and prior to idebenone use
- Have a genetic diagnosis for LHON for one of the following mtDNA mutations: G11778A, G3460A, T14484C

5.3 Case Record Survey SNT-CRS-002 exclusion criteria

- Any participation in an interventional clinical trial after the onset of symptoms
- Any other cause of visual impairment (e.g. glaucoma, diabetic retinopathy, AIDS related visual impairment, cataract, macular degeneration, etc.) or any active ocular disorder (uveitis, infections, inflammatory retinal disease, thyroid eye disease, etc.) during the data collection period

5.4 LEROS inclusion criteria

The following criteria should be assessed during Baseline Visit. If any does not apply, the patient must not be included in the study:

- Impaired visual acuity in affected eyes due to LHON
- No explanation for visual loss besides LHON
- Age ≥ 12 years
- Onset of symptoms ≤5 years prior to Baseline
- Confirmation of either G11778A, G3460A or T14484C LHON mtDNA (for the ITT population, not required for enrolment)
- Written informed consent obtained from the patient
- Ability and willingness to comply with study procedures and visits
- Women of Childbearing Potential (WCBP) who have a negative urine or serum pregnancy test at Baseline visit and who are willing to use a highly effective contraceptive measure and maintain it until treatment discontinuation.

5.5 LEROS exclusion criteria

The following criteria should be checked during Baseline Visit. If any applies, the patient must not be included in the study:

- Patient has provided natural history data to the Case Record Survey (SNT-CRS-002)
- Any previous use of idebenone
- Any other cause of visual impairment (e.g. glaucoma, diabetic retinopathy, AIDS related visual impairment, cataract, macular degeneration, etc.) or any active ocular disorder (uveitis, infections, inflammatory retinal disease, thyroid eye disease, etc.)
- Known history of clinically significant elevations (greater than 3 times the upper limit of normal) of AST, ALT or creatinine
- Patient has a condition or is in a situation which, in an investigator's opinion may put the patient at significant risk, may confound study results or may interfere significantly with the patient's participation in the study
- Participation in another clinical trial of any investigational drug within 3 months prior to Baseline
- Hypersensitivity to the active substance or to any of the following excipients (as listed in section 6.1 of Raxone SmPC): Lactose monohydrate, Microcrystalline cellulose, Croscarmellose sodium, Povidone K25, Magnesium stearate, Colloidal silica, Macrogol 3350, Poly(vinyl alcohol), Talc, Titanium dioxide, Sunset yellow FCF (E110).
- Women who are pregnant or have a positive pregnancy test at Baseline visit
- Women who are breastfeeding

6 Sample size considerations

The present study is designed to provide sufficient statistical power for a pre-planned indirect comparison of the primary endpoint against an external natural history control group.

As defined in the original LEROS study protocol (Version1.0, 29 January 2016, Section 9.6), the sample size calculation for LEROS assumed an expected 24% responder rate in the external natural history control group (combined data from SNT-IR-006 and SNT-CRS-002). The protocol defines a pre-planned check of the estimate of responder rate in the control group, once the enrolment of the new natural history study (SNT-CRS-002) has been completed. In case the responder rate in the control group is different from 24%, a sample size re-calculation using the updated control group estimate can be considered.

As SNT-CRS-002 study was completed (Clinical Study Report version 1.0 dated 15 July 2019), the data collected in the natural history studies has now been checked for the estimate of the responder rate. Based on the combined data from 175 eyes in the two natural history studies (SNT-IR-006 and SNT-CRS-002), the estimated responder rate is below 22%, i.e. lower than the initially expected rate of 24%. Although the determination of the exact responder rate for the natural history studies requires final data from the LEROS study for matching purposes, it is reasonable to assume that the responder rate of the natural history studies is not higher than 22%. The sample size for LEROS study is therefore re-calculated using the same methods as in the original protocol (Version1.0, 29 January 2016). In order to reach a power of 90% for the comparison of the responder rates between the natural history studies (assumed to be 22% instead of 24%) and the LEROS study (assumed to be 40%, as defined in the protocol), evaluable data from altogether 110 eyes from the LEROS study is required. In order to account for a drop-out rate of 30% (as defined in the protocol), at least 80 patients (equal to 160 eyes) will be enrolled to the LEROS study.

The sample size calculation was done with nQuery Advisor version 8.3.

For the sample size for the eye treated >1 year after the onset of symptoms and the corresponding external natural history control group, the aim is to collect VA data from same number of eyes as for the primary objective in order to compare the patients treated ≤1 and >1 year since the onset of symptoms and to compare patients treated with Raxone >1 year since the onset of symptoms with the corresponding natural history control group, assuming sufficient natural history control data can be collected to generate the natural history control group as described in 8.3.2.

7 Statistical hypotheses

The primary aim of the analysis is to show the superiority of Raxone® (patients treated ≤1 year after onset of symptoms) versus matching NH IDE naïve patients in proportion of eyes with clinically relevant recovery of VA from BL or 12 month maintenance of BL VA better than 1.0 logMAR

H_0: Raxone® (patients treated ≤1 year after onset of symptoms) is not superior to matching NH IDE naïve patients in proportion of eyes with CRR of VA from BL or 12 month maintenance of BL VA better than 1.0 logMAR

H_1: Raxone® (patients treated ≤1 year after onset of symptoms) is superior to matching NH IDE naïve patients in proportion of eyes with CRR of VA from BL or 12 month maintenance of BL VA better than 1.0 logMAR

A two-sided 95% confidence interval (CI) for the odds ratio (Raxone® versus NH IDE naïve) is used in the evaluation of the hypotheses. Superiority is shown if the lower limit of the two-sided 95% CI is greater than 1. This is equivalent to obtaining a p-value of less than 0.05 in the statistical test for the H_0.

8 Analysis sets

8.1 Safety Population

CRS-1 and CRS-2 did not collect any safety data.

Safety will only be assessed in LEROS patients.

The Safety Population will include all patients enrolled in the LEROS study, who received at least one dose of study medication. The Safety population will be used when evaluating safety variables.

8.2 Intent-To-Treat Population

There is no Intent-To-Treat (ITT) population defined for CRS-1 and CRS-2, and Natural History control set is defined in section 8.4.

ITT population will include all patients enrolled to LEROS which provide at least one post-BL VA assessment.

The ITT population in conjunction with the external NH control set will be used to evaluate efficacy endpoints defined in section 4.

8.3 Modified Intent-to-Treat Population

Modified Intent-to-Treat (mITT) population will include all patients enrolled to LEROS, who are carriers of only one of the three major LHON mtDNA (G11778A, G3460A or T14484C), received at least one dose of the study medication and provide at least one post-BL VA assessment.

The mITT population in conjunction with the external NH control set defined in section 8.4, will be used as a sensitivity analysis of the evaluation of efficacy endpoints defined in section 4.

8.4 Natural History control set

The Natural History (NH) external control set for LEROS study is constituted by the combined data from CRS-1 and CRS-2. In order to ensure comparability between populations, the following criteria, based on LEROS inclusion/exclusion (section 5.4 and 5.5) criteria and patients included in the ITT population, are required for subjects to be included in the external NH (Natural History) control set out of all patients included in CRS-1 and CRS-2:

- Carriers of only one of the three major LHON mtDNA (G11778A, G3460A or T14484C)
- Age ≥ 12 years;
- No other cause of visual impairment apart from LHON;
- Date of symptoms onset is defined (or at least month and year known);
- Onset of symptoms are less or equal to 5 years at BL visit;
- At least 2 VA assessments;
- VA assessments visit date must be defined (or at least month and year known).

The NH control set in conjunction with the ITT population will be used to evaluate efficacy endpoints defined in section 4.

8.5 LEROS and NH matched datasets

In order to optimize the comparability of patient data in the NH control set and the LEROS ITT population, subsets from the NH control set will be prospectively defined. These subsets will be matched separately for all the efficacy analyses using the matching rules defined in section 8.5.1 and 8.5.2.

A total of 8 datasets (as exemplified in Table 8-1) will be generated based on the matching algorithm (taking in consideration time since symptoms onset at BL and follow-up in analysis).

Table 8-1- LEROS and NH matched datasets

time since symptoms onset	follow-up (months)			
≤ 1 year	6	12	18	24
> 1 year	6	12	18	24

8.5.1 Matching algorithm for the primary endpoint

The external NH control set for the eyes with BL ≤1 year since onset of symptoms in the LEROS ITT population will be generated as follows (section 19.1 presents the matching algorithms using workflow diagrams and provides examples of matched BL and follow-up visits):

1. Since there is no treatment to be considered in the NH control set, in principle any VA observation at any time point after the onset of symptoms in any eye can be used as a BL for that eye. The primary outcome measure in LEROS is the VA at 12 months after BL. Therefore, using a "window" of ±3 months, any eye with a VA observation at any time point in the NH control set which has a follow-up VA assessment within

12±3 months will be retained for use as a possible BL observation. All such possible BL observations and subsequent VA assessments will form the NH control subset to be compared to the BL of LEROS.

2. In LEROS, the average time from the onset of symptoms to the start of Raxone® treatment will be calculated once all data is available. The start of the Raxone® treatment will be used as the BL in the statistical analyses.

3. For each VA observation retained in the NH control subset, the time from the onset of symptoms at each possible BL time point will be calculated for each eye.

4. For each eye in the NH control subset, the VA observation for which time since onset of symptoms is closest to the average time since onset of symptoms at BL calculated for LEROS (see 2) will be selected as the BL VA observation for that eye. Any eye in the NH control subset with a BL VA observation >6 months after the average time since onset of symptoms at BL in LEROS will be discarded. The resulting dataset will form the "final NH control subset" to be used for the primary endpoint. In this way, BL observations in the final NH control subset will be closely matched to the BL in the LEROS ITT population.

5. The change from BL in VA at 12±3 months for each eye in the final matched NH control subset will be compared to the change from BL visit to the visit scheduled at Month 12 in LEROS.

This algorithm will guarantee that the time from the onset of symptoms to the BL assessment is comparable between the final NH control dataset and LEROS.

The described process will be repeated for the secondary analyses applied for the 6, 18 and 24 month time points for the eyes with BL ≤1 year after the onset of symptoms.

8.5.2 Matching algorithm for the secondary endpoints

The external NH control set for the eyes with BL >1 year since onset of symptoms in the LEROS ITT population will be generated as follows (section 19.1 presents the matching algorithms using workflow diagrams):

1. All available eyes in the external NH control set will be considered, regardless of whether the eye in question was used in the control group for the primary objective or not.
2. All VA assessments >1 year after the onset of symptoms are considered as potential BL values.
3. If a potential BL value does not have a follow-up VA assessment within 12±3 months, it will be excluded.
4. The remaining potential BL values will be categorized in the following time since onset bins: >1-2 years, >2-3 years, >3-4 years or >4-5 years.
5. If there are several potential BL values for the same eye in the same bin, the eye with the BL value closest to the midpoint of the bin will be selected.
6. The selected BL value and the follow-up VA assessment within 12±3 months in the same eye will be included in the external NH control set for the eyes in the LEROS ITT population with BL >1 year after the onset of symptoms.

The described process will be applied for the 6, 18 and 24 month time points for the eyes with BL >1 year after the onset of symptoms.

It should be noted that different cuts (various BL with corresponding follow-up VA values) from the same eye can be used multiple times, both for the analysis of the primary endpoint (only once) and for the analysis of eyes with BL >1 year after the onset of symptoms (not more than once within each of the four bins of follow-up months defined in Table 1).

9 General statistical considerations

Continuous data will be summarized using the number of observations (n), mean, standard deviation (SD), standard error of the mean (SEM), median, 1st and 3rd quartile, minimum and maximum.

Categorical data will be presented in contingency tables with frequencies and percentages.

The listings will be generally sorted by center and patient number (and by visit, if applicable).

Graphical representations will be used as appropriate.

In general, apart from the safety population, data will be summarized by onset of symptoms

- ≤1 year after the onset of symptoms;
- >1 year after the onset of symptoms

, as defined in section 16.

In the statistical analyses a p-value less than 0.05 will be considered as statistically significant. If not stated otherwise, all tests will be performed as two sided tests and two sided 95% confidence intervals will be produced for the treatment differences.

CRS-1, CRS-2 and LEROS data will be presented in separate listings (one per study).

Unscheduled or repeat assessments in LEROS will not be included in summary tables, but will be included in listings.

Otherwise specified, listings will be sorted by patient number and visit date.

Deviations from the statistical plan will be reported in the clinical study report, including the rationale for the deviation.

9.1 Handling of drop-outs or missing data

Unless otherwise specified missing data will not be included in the analysis. LEROS data imputation rules are presented section 17.1. and NH data imputation rules are presented section 17.2)

In order to assess the impact of missing data, sensitivity analysis for the primary and secondary endpoints will be performed.

9.2 Interim analysis and data monitoring

No statistical interim analysis is planned.

To fulfil the regulatory obligations connected to the European Post Approval Commitment (PAES study), annual study reports are submitted yearly as part of the annual reassessment procedure where only study status and safety are discussed. No study endpoints or efficacy analysis are performed.

9.3 Examination of subgroups

Subgroup analyses will be performed in LEROS ITT population, considering time since symptoms onset in **most recent affected eye at BL**.

These subgroups will be analysed separately for patients ≤1 year and >1 year since symptoms onset in most recent affected eye at BL:

- LHON mtDNA mutations:
  - G11778A
  - G3460A
  - T14484C
  - Any other mutation
- Age at BL:
  - < 18 years;
  - ≥ 18 years and < 35 years;
  - ≥ 35 years
- Best VA Blindness Category (BC) at BL:
  - Off-chart: CF, HM, LP or NLP
  - Legally blind: 1.0 ≤ logMAR ≤1.68
  - Non-legally blind: logMAR < 1.0

10 Disposition

Patients' disposition will be presented separately for LEROS and Natural History (CRS-1 and CRS-2).

10.1 LEROS

The disposition of patients in LEROS will be summarized as presented in Figure 10.1.1 workflow diagram and Table 10-1.

Figure 10.1.1- LEROS subject disposition

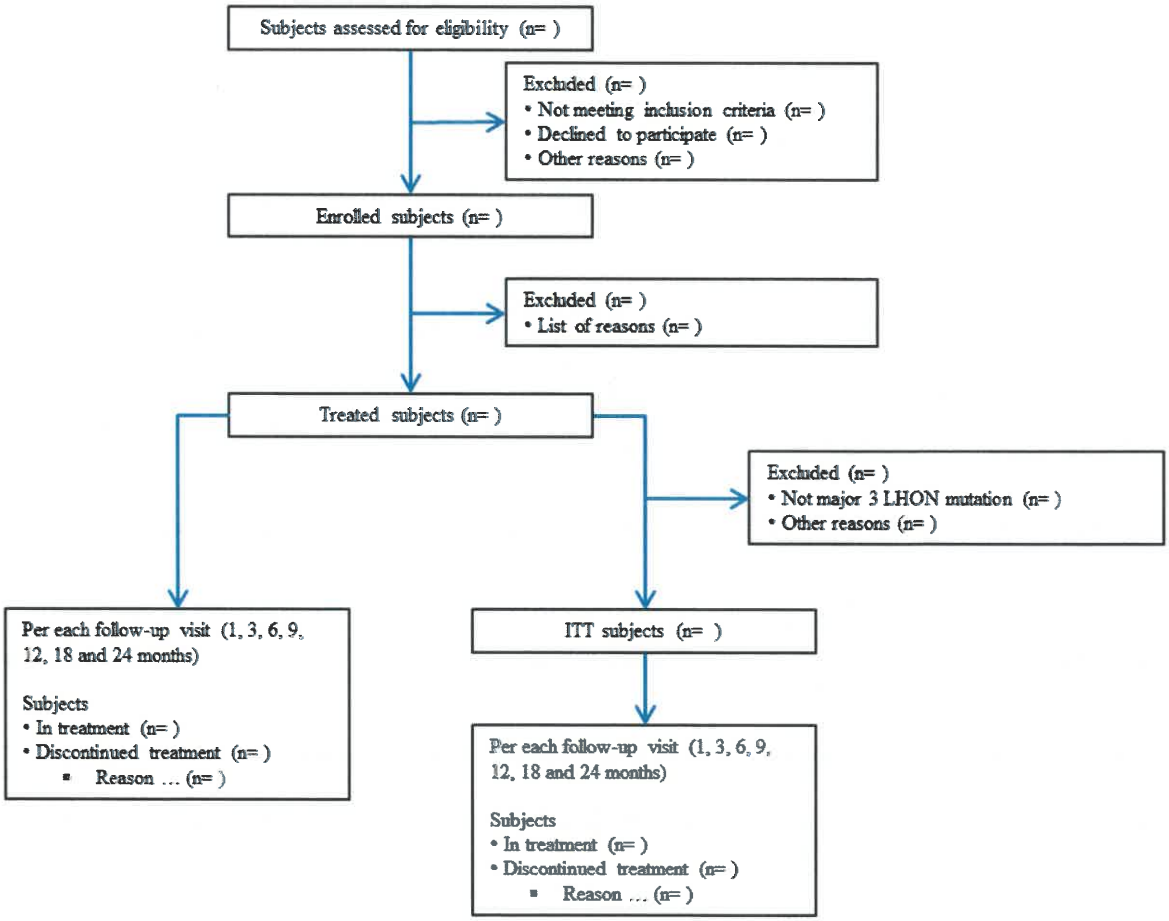


Table 10-1 LEROS duration of follow-up

	1 day	≥ 3 months	≥ 6 months	≥ 9 months	≥ 12 months	≥ 18 months	≥ 24 months
N	xx	xx	xx	Xx	Xx	xx	Xx
%	xx.x	xx.x	xx.x	xx.x	xx.x	xx.x	xx.x

Patients center disposition will be tabulated presenting the number and percentage of patients enrolled, patients in Safety population and patients in ITT population, in total and by year after the onset of symptoms. Information will be order by center identification and shown as presented in Table 10-2.

Table 10-2 LEROS patients by center

Patients by center		Onset ≤ 1 year[1]	Onset > 1 year[1]	Total of patients
Center	Patients Enrolled	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
	Safety population	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
	ITT population	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)

[1] Time since symptoms onset of most recent affected eye at baseline.

Listings: For enrolled patients it will be provided the following listings order by center and patient identification:

- date of inform consent, date of baseline visit, date of last recorded study visit, reason for end of study completion/discontinuation (if applicable, reason for study discontinuation), and date of symptoms onset of most recent affected eye at baseline;
- analysis population: Safety population, ITT (Intent-To-Treat population), modified ITT population;
- patients who did not meet the inclusion/exclusion criteria or confirmation of either G11778A, G3460A or T14484C was not present at baseline.

10.2 Natural History

The disposition of patients in Natural History (CRS-1 and CRS-2 patients) will be summarized as presented in Figure 10.2.1 workflow diagram and Table 10-3.

Figure 10.2.1- Natural History subject disposition

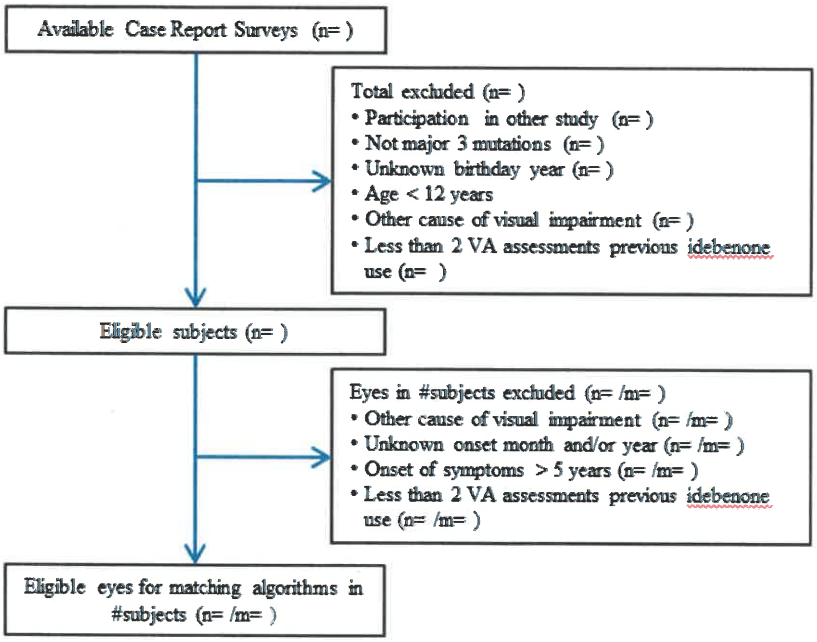


Table 10-3 Natural History duration of follow-up

	1 day	≥ 3 months	≥ 6 months	≥ 9 months	≥ 12 months	≥ 18 months	≥ 24 months	≥ 60 months
N	xx	xx	xx	xx	xx	xx	xx	xx
%	xx.x	xx.x	xx.x	xx.x	xx.x	xx.x	xx.x	xx.x

Patients center disposition will be tabulated presenting the number and percentage of patients per Case Report Survey. Information will be order by center identification as presented Table 10-4.

Table 10-4 Natural History patients by center

Patients by center	CRS-1	CRS-2	Total of patients
Center 1	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
Center 2	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
...	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)

Listings: It will be provided a list with date of informed consent, date of first recorded visit and date of last recorded visit, order by report survey, center and patient identification.

11 Data Collection and Analysis

11.1 Demographic and other Baseline characteristics

Variables: Table 11-1 shows which variables to analyse for the different analysis data sets.

Analysis method: Demographic and BL characteristics will be analysed by means of descriptive statistics. The start of the Raxone® treatment will be used as the BL (LEROS ITT).

Table shell structure is presented section 19.2.

Table 11-1 - Demographic and BL characteristics by analysis sets and subgroups

	Variable Type[1]	Analysis sets					
		LEROS mITT / NH matched sets[2]	Safety population	LEROS ITT	mtDNA subgroups[3]	Age at BL subgroups[3]	Best VA at BL subgroups[3]
Demographics							
Gender	N	X	X	X	X	X	X
Child bearing potential	N	-	X	X	X	X	X
Mutation	N	X	X	X	X	X	X
Race	N	-	X	X	X	X	X
Height	C	-	X	X	X	X	X
Weight	C	-	X	X	X	X	X
Age at BL	C	X	X	X	X	X	X
Baseline characteristics							
Age at 1st symptoms onset [4]	C	X	-	X	X	X	X
Months since 1st symptoms onset	C	X	-	X	X	X	X
Months since most recent symptoms onset	C	X	-	X	X	X	X

Number of symptomatic eyes per patient	O	X	-	X	X	X	X
Eye with 1st symptoms onset	N	X	-	X	X	X	X
Delta onset (months)[5]	C	X	-	X	X	X	X

[1] Variable Type: nominal (N), ordinal (O), continuous (C); [2] Matched data sets (as defined in section 8.5) used in the calculation of 12 and 24 months endpoints evaluation; [3] Subgroups are to be analysed as defined in 9.3; [4] Age calculated as date of baseline visit minus date of birth, divided by 365.25; [5] Patients whose eyes have the same date of symptom onset will not be accounted

Listings: LEROS Safety population race, gender, weigh, height, age at baseline and LHON mtDNA will be listed, order by patient unique identification number.

For Natural History patients gender, age at baseline and LHON mtDNA will be listed, order by report survey , center and patient identification.

11.2 Visual Acuity

VA at BL, follow-up (month 6, 12, 18 and 24) and at nadir (as defined in section 16) will be analysed by means of descriptive statistics.

VA variables to analyse for the different analysis data sets and subgroups are described in Table 11-2 to Table 11-4. Table shells structure is presented in section 19.2.

Listings:

LEROS Safety population date of visit, type of visit (baseline, follow-up month or unscheduled), left eye logMAR, left eye semi-quantitative, right eye logMAR and right eye semi-quantitative will be listed, order by center, patient identification and date of visit.

For Natural History patients date of visit, left eye logMAR, left eye semi-quantitative, right eye logMAR and right eye semi-quantitative will be listed, order by report survey, center, patient identification and date of visit.

11.2.1 Visual Acuity at BL

Baseline descriptive statistics will be presented both for eyes and patients. Variables to be analysed by Month 12 and 24 visits are presented in Table 11-2.

Table 11-2 – Visual Acuity at BL by analysis sets and subgroups

VA at BL	Variable Type[1]	Analysis sets				
		LEROS mITT / NH matched sets[3]	LEROS ITT	mtDNA subgroups[3]	Age at BL subgroups[3]	Best VA at BL subgroups[3]
Based in total of Patients						
Best VA by blindness category	O	X	X	X	X	X
Best VA (logMAR)	C	X	X	X	X	X
Based in total of Eyes						
Months since symptoms onset	C	X	X	X	X	X
Eyes VA by blindness category	O	X	X	X	X	X
Eyes VA (logMAR)	C	X	X	X	X	X

[1] Variable Type: nominal (N), ordinal (O), continuous (C); [2] Matched data sets (as defined in section 8.5) used in the calculation of 12 and 24 months endpoints evaluation; [3] Subgroups are to be analysed as defined in 9.3;

11.2.2 Visual Acuity at Nadir

Nadir (as defined in section 16) descriptive statistics will only be presented for eyes. Variables to be analysed are presented in Table 11-3.

Time to Nadir will also be presented using Kaplan-Meier estimate and Kaplan-Meier curves (nadir as event and study exit as censor). Table shell's structure is presented in section 19.2.

Table 11-3 – Nadir at follow-up visit by analysis sets and subgroups

VA at Nadir	Variable Type[1]	Analysis sets			
		LEROS ITT	mtDNA subgroups[2]	Age at BL subgroups[2]	Best VA at BL subgroups[2]
Eyes as base					
Months since BL at Nadir	C	X	X	X	X
VA by blindness category at Nadir	O	X	X	X	X
VA logMAR at Nadir	C	X	X	X	X

[1] Variable Type: nominal (N), ordinal (O), continuous (C); [2] Subgroups are to be analysed as defined in 9.3;

11.2.3 Visual Acuity at follow-up

VA at Month 6, 12, 18 and 24 visits descriptive statistics will be presented both for eyes and patients. Variables to be analysed are presented in Table 11-4. Each follow-up visit will be presented in separate tables. Table shell's structure is presented in section 19.2.

Table 11-4 –Visual Acuity at follow-up visit (Month 6, 12, 18 and 24) by analysis sets and subgroups

VA at Month XX	Variable Type[1]	Analysis sets				
		LEROS mITT / NH matched sets[2]	LEROS ITT	mtDNA subgroups[3]	Age at BL subgroups[3]	Best VA at BL subgroups[3]
Based in total of Patients						
Best VA by blindness category	O	X	X	X	X	X
Best VA (logMAR)	C	X	X	X	X	X
Change in Best logMAR compared to BL	C	X	X	X	X	X
Based in total of Eyes						
Eyes VA by blindness category	O	X	X	X	X	X
Eyes VA (logMAR)	C	X	X	X	X	X
Change in Eyes logMAR compared to BL	C	X	X	X	X	X

[1] Variable Type: nominal (N), ordinal (O), continuous (C); [2] Matched data sets (as defined in section 8.5) used in the calculation of 12 and 24 months endpoints evaluation; [3] Subgroups are to be analysed as defined in 9.3;

11.3 Concomitant medication/treatment

In CRS-1 and CRS-2 no concomitant medication/treatment information was collected.

The concomitant medication data in LEROS trial will be summarized descriptively for the Safety population. All medications will be classified using the Anatomical Therapeutic Chemical (ATC) classification codes and preferred drug names from the World Health Organization Drug Dictionary (WHO-DD).

Data will be tabulated presenting the number and percentage of patients with receiving at least one medication; patient frequencies and percentages on the ATC Level 1, ATC Level 2, and preferred drug names as exemplified in Table 11-5.

The tables will be sorted by overall descending frequency of ATC Level(s) and then, within an ATC Level, by overall descending frequency of preferred drug name.

Table 11-5 LEROS Concomitant Medication

	n (%)
Number of patients receiving concomitant medication	xx (xx.x%)
ATC Level 1	xx (xx.x%)
- ATC Level 2	xx (xx.x%)
• drug name	xx (xx.x%)

11.4 Medical history

LEROS Safety population and CSR-2 patients will be summarized descriptively (separately); and data will be coded using the Medical Dictionary for Regulatory Activities (MedDRA).

The medical history will be tabulated presenting the number and percentage of patients with at least one medical history item; patient frequencies and percentages on the System Organ Class (SOC) and Preferred Term (PT) levels as exemplified in Table 11-6. The table will be sorted by overall descending frequency of SOC and then, within a SOC, by overall descending frequency of PT.

Table 11-6 LEROS and CSR-2 Medical History

	n (%)
Number of patients with at least one medical history term	xx (xx.x%)
SOC	xx (xx.x%)
- PT	xx (xx.x%)

Listings:

CRS-1 data was not collected. It will be provided a listing presenting the investigator's comments at baseline, order by center and patient identification.

It will be provided a SOC and PT listing for LEROS Safety population and CSR-2 patients (separately), order by center, patient identification, SOC and PT.

12 Analysis of efficacy

The criteria of inclusion/exclusion for the pre-planned indirect comparison of the primary endpoint against an external natural history control group, where it is only considered who are carriers of only one of the three major LHON mtDNA (G11778A, G3460A or T14484C). Due this criterion, efficacy analysis where there is an indirect comparison to the external natural history control group, will be based on the mITT population.

12.1 Primary endpoint

Endpoint: Proportion of eyes with clinically relevant recovery (CRR) of visual acuity from Baseline (BL) or in which BL VA better than 1.0 logMAR was maintained at Month 12 in patients treated with Raxone® ≤1 year after the onset of symptoms, compared to matching external natural history control group. CRR definition is presented in section 16.

Analysis sets: Primary endpoint analysis will use all LEROS mITT eyes that are ≤1 year since onset of symptoms at BL and have the Month 12 assessment. External NH control set is generated as described in section 8.5.1 (Matching algorithm for the primary endpoint), using VA assessments within 12±3 months from BL.

Analysis method: The primary endpoint will be analysed using a logistic regression model. The binary response will be used as the dependent variable. The independent variables include the treatment group (Raxone® treated patients versus untreated control group), gender (male, female) and mutation group (G11778A, G3460A, T14484C) as fixed factors.

The difference between the Raxone® treated patients versus NH group will be estimated with odds ratio, two-sided 95% confidence intervals and two-sided p-values

```
proc logistic;
   class treatment gender mutation / param=ref;
    model CRB(event='1')  = treatment mutation gender / link=logit clodds=pl;
   oddsratio treatment;
run;
```

12.1.1 Sensitivity analysis of the primary endpoint

Imputation of missing data

To assess the impact of missing data, sensitivity analysis using the multiple imputation approach will be applied. All missing data will be assumed to be missing at random (MAR). The imputation will consider and use only LEROS data, since the matched external natural history control group is not longitudinal data and does not include any missing data. The VA assessments from all visits up to Month 12 will be used. First the MCMC methodology will be applied to impute the data to obtain monotonously missing data. Then imputation will be completed using regression imputation for the monotonously missing data. CRB will be defined based on the imputed VA data. Analysis model will be run for the imputed 12-month data by imputation and SAS procedure *mianalyze* will be used to calculate the combined results.

SAS code to perform multiple imputation on missing visual acuity results assuming MAR (values: Raxone® treated patients = 1 ; NH = 0)

```
/*    STEP 1: LEROS data imputation*/
/*impute non-monotonously missing observations */
proc mi data=visual_acuity out=visual_acuite_monotone nimpute=1000 seed=xxx;
   var v2logMAR v3logMAR v4logMAR v5logMAR v6logMAR;
   mcmc chain=multiple impute=monotone;
run;

/*use regression imputation to complete MAR imputation*/
proc mi data= visual_acuite_monotone out=visual_acuite_imputed nimpute=1 seed=xxx;
   var v2logMAR v3logMAR v4logMAR v5logMAR v6logMAR;
   monotone regression;
run;

/*    STEP 2: calculation of visit 6 CRB from baseline for imputed values
            visit 6 CRB from baseline should be calculated with the same SAS code
            used for the main analysis of the primary endpoint

            input dataset: visual_acuite_imputed;
```

```
                calculate LEROS visit 6 CRB;
                output dataset: leros_crb_imputed
*/

/*    STEP 3:  repeat 1000 times Natural History data used the main analysis of the
                primary endpoint and join with LEROS data set which has the imputed CRB calculation*/

data natural_history_repeated;
   set natural_history_analysis_data;
      do _Imputation_ = 1 to 1000;
         output;
      end;
   run;

data all_data_joined;
         set leros_crb_imputed natural_history_repeated;
run;

/*    STEP 4:  applying logistic model over the new imputed CRB data*/
proc sort data= all_data_joined; by Imputation; run;

proc logistic;
    by _Imputation_;
    class treatment gender mutation / param=ref;
    model CRB(event='1')  = treatment gender mutation / link=logit clodds=pl;
    ods output ParameterEstimates = ParameterEstimates;
    ods output CLOddsPL = CLOddsPL;
run;

/*    STEP 5: calculate the logistic analysis combined results*/
proc mianalyze parms(classvar=classval)= ParameterEstimates.;
    modeleffects treatment gender mutation;
run;
```

```
/*    STEP 6: calculate the combined results odds ratio */
/* Transform estimates */
data calculated_odds_ratio_1;
   label or_value = 'Log odds ratio value'
         or_stderr = 'Log odds ratio SE';

   set CLOddsPL;
      or_value = log(OddsRatioEst);
      or_stderr= (log(uppercl)-log(lowercl))/(2*1.96);
run;
/*   Combine transformed estimates */
proc sort data= calculated_odds_ratio_1; by Effect; run;
proc mianalyze data= calculated_odds_ratio_1;
        by Effect;
        modeleffects or_value;
        stderr or_stderr;
        ods output ParameterEstimates = calculated_odds_ratio_2;
      run;

/*    Back-transform combined values */
data calculated_odds_ratio_3;
   label estimate_back = 'Odds ratio'
         lcl_back = 'LCL Odds ratio'
         ucl_back = 'UCL Odds ratio';

        set calculated_odds_ratio_2;
        estimate_back = exp(estimate); *pooled odds ratio;
        lcl_back=estimate_back*exp(-1.96*stderr); *pooled lower limit;
        ucl_back=estimate_back*exp(+1.96*stderr); *pooled upper limit;
      run;
```

Through inverse probability of treatment

Propensity score will be calculated by means of logistic regression with treatment as dependent variable. Independent variables include gender (male, female), mutation group (G11778A, G3460A, T14484C), age, time since symptoms onset in months and VA at BL (logMAR) as factors.

The same primary model as described above will be applied with the inverse probability weight.

SAS code to calculate the propensity score (values: Raxone® treated patients = 1 ; NH = 0)

```
proc logistic data=CRB_dataset;
   class gender mutation / param=ref;
   model treatment(event='1') = gender mutation age time_onset bl_va;
   output out=psdataset pred=ps;
run;

data analysis_dataset;
   set psdataset;
   if treatment = 1 then ps_weight = 1/ps;
   else if treatment = 0 then ps_weight = 1/(1 – ps);
run;
```

SAS code to proceed with the endpoint regression analysis

```
proc logistic data= analysis_dataset;
   class treatment gender mutation / param=ref;
    model CRB(event='1') = treatment gender mutation / link=logit clodds=pl;
   oddsratio treatment;
   weight ps_weight;
run;
```

Effect of the size of time window used in the matching

The effect of the size of time window used in the matching algorithm can be explored rerunning the algorithm and primary analysis using different time windows

- VA assessments within 12±3.5 months from BL
- VA assessments within 12±4 months from BL

The same primary model as described above will be applied.

12.2 Efficacy Secondary Endpoints

Endpoint: Components of the primary endpoint: proportion of eyes with CRR of VA from BL at Month 12 compared to matching external NH control group

Analysis method: This endpoint will be analysed with methods similar as for analysing the primary endpoint as defined in section The criteria of inclusion/exclusion for the pre-planned indirect comparison of the primary endpoint against an external natural history control group, where it is only considered who are carriers of only one of the three major LHON mtDNA (G11778A, G3460A or T14484C). Due this criterion, efficacy analysis where there is an indirect comparison to the external natural history control group, will be based on the mITT population.

Primary endpoint 12.1.

Endpoint: Components of the primary endpoint: proportion of eyes in which BL VA better than 1.0 logMAR was maintained at Month 12 compared to matching external NH control group

Analysis method: This endpoint will be analysed with methods similar as for analysing the primary endpoint as defined in section The criteria of inclusion/exclusion for the pre-planned indirect comparison of the primary endpoint against an external natural history control group, where it is only considered who are carriers of only one of the three major LHON mtDNA (G11778A, G3460A or T14484C). Due this criterion, efficacy analysis where there is an indirect comparison to the external natural history control group, will be based on the mITT population.

Primary endpoint 12.1.

Endpoint: Proportion of eyes in patients treated with Raxone® >1 year after the onset of symptoms with CRR of VA from BL or in which BL VA better than 1.0 logMAR was maintained at Month 12 compared to external NH control group

Analysis method: This endpoint will be analysed with methods similar as for analysing the primary endpoint as defined in section The criteria of inclusion/exclusion for the pre-planned indirect comparison of the primary endpoint against an external natural history control group, where it is only considered who are carriers of only one of the three major LHON mtDNA (G11778A, G3460A or T14484C).

Due this criterion, efficacy analysis where there is an indirect comparison to the external natural history control group, will be based on the mITT population.

Primary endpoint 12.1.

Endpoint: Proportion of eyes and patients treated with Raxone® ≤1 year after the onset of symptoms with CRR of VA from BL or in which BL VA better than 1.0 logMAR was maintained following 6, 18 and 24 months of treatment with Raxone® compared to matching external NH control group

Analysis method: This endpoint will be analysed separately for each follow-up with methods similar as for analysing the primary endpoint as defined in section The criteria of inclusion/exclusion for the pre-planned indirect comparison of the primary endpoint against an external natural history control group, where it is only considered who are carriers of only one of the three major LHON mtDNA (G11778A, G3460A or T14484C). Due this criterion, efficacy analysis where there is an indirect comparison to the external natural history control group, will be based on the mITT population.

Primary endpoint 12.1.

Endpoint: Proportion of eyes/patients treated with Raxone® ≤1 year after the onset of symptoms with "Off-chart" VA at BL in whom VA improves to better than 1.60 logMAR by Month 6, 12, 18 and 24

Analysis method: This endpoint will be analysed by means of descriptive statistics in the LEROS ITT population and presented in contingency tables with frequencies and percentages as illustrated in section 19.2.

Endpoint: Proportion of eyes/patients treated with Raxone® >1 year after the onset of symptoms with "Off-chart" VA at BL in whom VA improves to better than 1.60 logMAR by Month 6, 12, 18 and 24

Analysis method: This endpoint will be analysed by means of descriptive statistics in the LEROS ITT population and presented in contingency tables with frequencies and percentages as illustrated in section 19.2.

Endpoint: Proportion of eyes/patients treated with Raxone® ≤1 year after the onset of symptoms with VA in the categories of better than 1.0 logMAR, 1.0 to 1.68 logMAR and above 1.68 logMAR at each assessment time point up to Month 24

Analysis method: This endpoint will by means of descriptive statistics in the LEROS ITT population and presented in contingency tables with frequencies and percentages as illustrated in section 19.2.

Endpoint: Proportion of eyes/patients treated with Raxone® >1 year after the onset of symptoms with VA in the categories of better than 1.0 logMAR, 1.0 to 1.68 logMAR and above 1.68 logMAR at each assessment time point up to Month 24

Analysis method: This endpoint will by means of descriptive statistics in the LEROS ITT population and presented in contingency tables with frequencies and percentages as illustrated in section 19.2.

12.3 Additional efficacy analysis

Outcome: Proportion of eyes and patients treated with Raxone® ≤1 year after the onset of symptoms with Clinically Relevant Worsening (CRW) of VA from BL at Month 12 compared to matching external NH control group.

Analysis method: This outcome will by means of descriptive statistics

Outcome: Proportion of eyes and patients treated with Raxone® >1 year after the onset of symptoms with Clinically Relevant Worsening (CRW) of VA from BL at Month 12 compared to matching external NH control group.

Analysis method: This outcome will by means of descriptive statistics

Outcome: Proportion of eyes and patients treated with Raxone® ≤1 year after the onset of symptoms with Clinically Relevant Worsening (CRW) of VA from BL at Month 24 compared to matching external NH control group.

Analysis method: This outcome will by means of descriptive statistics

Outcome: Proportion of eyes and patients treated with Raxone® >1 year after the onset of symptoms with Clinically Relevant Worsening (CRW) of VA from BL at Month 24 compared to matching external NH control group.

Analysis method: This outcome will by means of descriptive statistics

Outcome: Eyes with CRR (and related information, Table 12-1) from BL at 24 months considering time symptoms onset in the most recent affected eye at BL.

Analysis method: This outcome will be analysed by means of descriptive statistics and a complementary analysis using Kaplan-Meier method in the LEROS ITT population and in the subgroups specified in section 9.3. Survival analysis will use CRR as an event, months in treatment at CRR as time of event, and months at last assessment during treatment as censoring. It will be presented the cumulative percent curve of CRR using months since BL at CRR as variable. Inverse Kaplan-Meier curves of time (in months) to CRR (with number of eyes at risk and censored at 6, 12, 18 and 24 months) will be presented.

Table 12-1 – LEROS ITT eyes with CRR from BL at 24 months

Eyes with CRR from BL	Variable Type[1]	Analysis sets			
		LEROS ITT	mtDNA subgroups[4]	Age at BL subgroups [4]	Best VA BC at BL subgroups [4]
CRR	N	X	X	X	X
Months in treatment since BL at CRR[2]	C	X	X	X	X
Time in treatment at CRR by follow-up[2,3]	N	X	X	X	X
Change in logMAR compared to BL at CRR[2]	C	X	X	X	X
Change in logMAR compared to BL at 24 months[2]	C	X	X	X	X
CRR Kaplan-Meier analysis	-	X	X	X	X

[1] Variable Type: nominal (N), ordinal (O), continuous (C); [2] Only eyes with CRR from BL should be accounted; [3] Time in treatment categories defined in section 16; [4] Subgroups are to be analysed as defined in 9.3;

Outcome: Patients with CRR from BL at 24 months (and related information, Table 12-2) considering time symptoms onset in the most recent affected eye at BL.

Analysis method: This outcome will be analysed by means of descriptive statistics and a complementary analysis using Kaplan-Meier method in the LEROS ITT population and in the subgroups specified in section 9.3. Survival analysis will use CRR as an event, months in treatment at CRR as time of event, and months at last assessment during treatment as censoring. It will be presented the cumulative percent curve of CRR using months since BL at CRR as variable. Inverse Kaplan-Meier curves of time (in months) to CRR (with number of patients at risk and censored at 6, 12, 18 and 24 months) will be presented.

Table 12-2 – LEROS ITT patients with CRR from BL at 24 months

Patients with CRR from BL	Variable Type[1]	Analysis sets			
		LEROS ITT	mtDNA subgroups [4]	Age at BL subgroups [4]	Best VA BC at BL subgroups [4]
CRR	N	X	X	X	X
Months in treatment since BL at CRR[2]	C	X	X	X	X
Time in treatment at CRR by follow-up[2,3]	N	X	X	X	X
Change in logMAR compared to BL at 1st CRR[2]	C	X	X	X	X
Best change in logMAR compared to BL at 24 months[2]	C	X	X	X	X
CRR Kaplan-Meier analysis	-	X	X	X	X

[1] Variable Type: nominal (N), ordinal (O), continuous (C); [2] Only eyes with CRR from BL should be accounted; [3] Time in treatment categories defined in section 16; [4] Subgroups are to be analysed as defined in 9.3;

Outcome: Eyes with CRR (and related information, Table 12-1) from Nadir (as defined in section 16) at 24 months considering time symptoms onset in the most recent affected eye at BL.

Analysis method: This outcome will be analysed by means of descriptive statistics and a complementary analysis using Kaplan-Meier method in the LEROS ITT population and in the subgroups specified in section 9.3. Survival analysis will use CRR as an event, months in treatment at CRR as time of event, and months at last assessment during treatment as censoring. It will be presented the cumulative percent curve of CRR using months since BL at CRR as variable. Inverse Kaplan-Meier curves of time (in months) to CRR (with number of eyes at risk and censored at 6, 12, 18 and 24 months) will be presented.

Table 12-3 – LEROS ITT eyes with CRR from Nadir at 24 months

Eyes with CRR from Nadir	Variable Type[1]	Analysis sets			
		LEROS ITT	mtDNA subgroups [4]	Age at BL subgroups [4]	Best VA BC at BL subgroups [4]
CRR	N	X	X	X	X

Months in treatment since BL at CRR[2]	C	X	X	X	X
Time in treatment at CRR by follow-up[2,3]	N	X	X	X	X
Change in logMAR compared to BL at CRR[2]	C	X	X	X	X
Change in logMAR compared to BL at 24 months[2]	C	X	X	X	X
CRR Kaplan-Meier analysis	-	X	X	X	X

[1] Variable Type: nominal (N), ordinal (O), continuous (C); [2] Only eyes with CRR from Nadir should be accounted; [3] Time in treatment categories defined in section 16; [4] Subgroups are to be analysed as defined in 9.3;

Outcome: Patients with CRR from Nadir (as defined in section 16) at 24 months (and related information, Table 12-2) considering time symptoms onset in the most recent affected eye at BL.

Analysis method: This outcome will be analysed by means of descriptive statistics and a complementary analysis using Kaplan-Meier method in the LEROS ITT population and in the subgroups specified in section 9.3. Survival analysis will use CRR as an event, months in treatment at CRR as time of event, and months at last assessment during treatment as censoring. It will be presented the cumulative percent curve of CRR using months since BL at CRR as variable. Inverse Kaplan-Meier curves of time (in months) to CRR (with number of patients at risk and censored at 6, 12, 18 and 24 months) will be presented.

Table 12-4 – LEROS ITT patients with CRR from Nadir at 24 months

Patients with CRR from Nadir	**Variable Type[1]**	**Analysis sets**			
		LEROS ITT	mtDNA subgroups[4]	Age at BL subgroups[4]	Best VA at BL subgroups[4]
CRR	N	X	X	X	X
Months in treatment at CRR[2]	C	X	X	X	X
Time in treatment at CRR by follow-up[2,3]	N	X	X	X	X
Change in logMAR compared to BL at 1st CRR[2]	C	X	X	X	X

Best change in logMAR compared to BL at 24 months[2]	C	X	X	X	X
CRR Kaplan-Meier analysis	-	X	X	X	X

[1] Variable Type: nominal (N), ordinal (O), continuous (C); 2 Only eyes with CRR from Nadir should be accounted; [3] Time in treatment categories defined in section 16; [4] Subgroups are to be analysed as defined in 9.3;

Outcome: Eyes with CRR and eyes that maintained CRR from Baseline after treatment discontinuation (Table 12-5) considering time symptoms onset in the most recent affected eye at BL.

Analysis method: This outcome will be analysed by means of descriptive statistics, accounting only eyes with a VA assessment at Visit 9. For the calculation maintenance of CRR, only eyes with CRR from Baseline at 24 months will be accounted.

Outcome: Patients with CRR and patients who maintained CRR from Baseline after treatment discontinuation (Table 12-5) considering time symptoms onset in the most recent affected eye at BL.

Analysis method: This outcome will be analysed by means of descriptive statistics, accounting only patients with a VA assessment at Visit 9. For the calculation maintenance of CRR, only patients with CRR from Baseline at 24 months will be accounted.

Table 12-5 – LEROS ITT CRR from Baseline and CRR from Baseline maintenance post-treatment discontinuation

CRR from Baseline	Variable Type[1]	Analysis sets			
		LEROS ITT	mtDNA subgroups[2]	Age at BL subgroups[2]	Best VA at BL subgroups[2]
Eyes with CRR	N	X	X	X	X
Eyes with maintenance of CRR	N	X	X	X	X
Patients with CRR	N	X	X	X	X
Patients with maintenance of CRR	N	X	X	X	X

[1] Variable Type: nominal (N), ordinal (O), continuous (C); [4] Subgroups are to be analysed as defined in 9.3;

Outcome: Eyes with CRR and eyes that maintained CRR from Nadir (as defined in section 16) after treatment discontinuation (Table 12-6) considering time symptoms onset in the most recent affected eye at BL.

Analysis method: This outcome will be analysed by means of descriptive statistics, accounting only eyes with a VA assessment at Visit 9. For the calculation maintenance of CRR, only eyes with CRR from Nadir at 24 months will be accounted.

Outcome: Patients with CRR and patients who maintained CRR from Nadir (as defined in section 16) after treatment discontinuation (Table 12-6) considering time symptoms onset in the most recent affected eye at BL.

Analysis method: This outcome will be analysed by means of descriptive statistics, accounting only patients with a VA assessment at Visit 9. For the calculation maintenance of CRR, only patients with CRR from Nadir at 24 months will be accounted.

Table 12-6 – LEROS ITT CRR from Nadir and CRR from Baseline maintenance post-treatment discontinuation

CRR from Nadir	Variable Type[1]	Analysis sets			
		LEROS ITT	mtDNA subgroups[2]	Age at BL subgroups[2]	Best VA at BL subgroups[2]
Eyes with CRR	N	X	X	X	X
Eyes with maintenance of CRR	N	X	X	X	X
Patients with CRR	N	X	X	X	X
Patients with maintenance of CRR	N	X	X	X	X

[1] Variable Type: nominal (N), ordinal (O), continuous (C); [4] Subgroups are to be analysed as defined in 9.3;

12.4 Sensitivity analysis due COVID-19 pandemic

Due to the COVID-19 pandemic, study visits can be rescheduled within

- Visits 6 and 7 it is 90 days,
- Visit 9 it is 60 days.
- Visit 8 if

- ≤60 days later than planned visit per protocol and ≤30 days no study medication intake at rescheduled visit 8 then visit 8 was performed
- >60 days later than planned visit per protocol and >30 days no study medication intake at rescheduled visit 8 then visit 9 was performed and visit 8 was skipped

of originally scheduled visit date.

The impact of the rescheduling or visits missed due to COVID-19 will be investigated with a sensitivity analysis using the approach proposed by Meyer et al (2020).

At the time of 12 months follow-up (Visit 6) only one patient missed the visit due to COVID-19 pandemic. Therefore, sensitivity analysis will be used in mITT population on the 24 months data (Visit 8).

Post-treatment data (Visit 9) will not be imputed.

All missing data will be categorized as being missing due to COVID-19 or due to other causes. In the first step, the missing data due to COVID-19 will be imputed using the assumption of MAR. In the second step, the remaining missing data will be imputed using the assumption of MNAR. For observations MNAR, last-observation-carried-forward will be applied, using only data collected from month 12 visit (Visit 6) onwards. For patients with no data in this time frame, the data from the follow-up visit will be used as the 24 month assessment. This sensitivity analysis will be repeated for a dataset where the Visual Acuity assessments that were delayed by more than 30 days from the scheduled visit date will be set as missing and included in the category of missed assessments due to COVID-19.

For the missing data assuming MAR, the multiple imputation approach will be applied. The imputation will consider and use only LEROS data, since the matched external natural history control group is not longitudinal data and does not include any missing data. As the disease does not progress linearly, only the 12 month (Visit 6) and 18 month (Visit 7) data will be used for the imputations. First the MCMC methodology will be used to impute the data to obtain monotonously missing data. Then imputation will be completed using regression imputation for the monotonously missing data. Analysis model will be run for the imputed data by imputation and SAS procedure *mianalyze* will be used to calculate the combined results.

SAS code to perform multiple imputation on missing visual acuity results assuming MAR:

```
/*    STEP 1: LEROS data imputation*/
/*impute non-monotonously missing observations */
proc mi data=visual_acuity out=visual_acuite_monotone nimpute=1000 seed=xxx;
  var logMAR_v6 logMAR_v7 logMAR_v8;
```

```
   mcmc chain=multiple impute=monotone;
run;


/*use regression imputation to complete MAR imputation*/
proc mi data= visual_acuite_monotone out=visual_acuite_imputed nimpute=1 seed=xxx;
   var logMAR_v6 logMAR_v7 logMAR_v8;
   monotone regression;
run;


/*    STEP 2: calculation of visit 8 CRB from baseline for imputed values
                visit 8 CRB from baseline should be calculated with the same SAS code
                used for the main analysis of the primary endpoint but use visit 8 as main variable

                input dataset: visual_acuite_imputed;
                calculate LEROS visit 8 CRB;
                output dataset: leros_crb_imputed
*/

/*    STEP 3:  repeat 1000 times Natural History data used the main analysis of the
                 primary endpoint and join with LEROS data set which has the imputed CRB calculation*/

data natural_history_repeated;
   set natural_history_analysis_data;
      do _Imputation_ = 1 to 1000;
         output;
      end;
   run;

data all_data_joined;
         set leros_crb_imputed natural_history_repeated;
run;

/*    STEP 4:  applying logistic model over the new imputed CRB data*/
proc sort data= all_data_joined; by Imputation; run;

proc logistic;
    by _Imputation_;
```

```
    class treatment gender mutation / param=ref;
    model CRB(event='1')  = treatment gender mutation / link=logit clodds=pl;

    ods output ParameterEstimates = ParameterEstimates;
    ods output CLOddsPL = CLOddsPL;
run;

/*    STEP 5: calculate the logistic analysis combined results*/
proc mianalyze parms(classvar=classval)= ParameterEstimates.;
    modeleffects treatment gender mutation;
run;

/*    STEP 6: calculate the combined results odds ratio */
/* Transform estimates */
data calculated_odds_ratio_1;
    label or_value = 'Log odds ratio value'
          or_stderr = 'Log odds ratio SE';

    set CLOddsPL;
       or_value = log(OddsRatioEst);
       or_stderr= (log(uppercl)-log(lowercl))/(2*1.96);
run;
/*    Combine transformed estimates */
proc sort data= calculated_odds_ratio_1; by Effect; run;
proc mianalyze data= calculated_odds_ratio_1;
          by Effect;
          modeleffects or_value;
          stderr or_stderr;
          ods output ParameterEstimates = calculated_odds_ratio_2;
       run;
```

```
/*   Back-transform combined values */
     data;
   label estimate_back = 'Odds ratio'
         lcl_back = 'LCL Odds ratio'
         ucl_back = 'UCL Odds ratio';

        set calculated_odds_ratio_2;
        estimate_back = exp(estimate); *pooled odds ratio;
        lcl_back=estimate_back*exp(-1.96*stderr); *pooled lower limit;
        ucl_back=estimate_back*exp(+1.96*stderr); *pooled upper limit;
     run;
```

12.5 Efficacy analysis listings

Listings: It be provided a listing with date of visit, visit type (baseline, follow-up month), eye (Left/Right), logMAR VA at the visit, logMAR VA at baseline, difference in logMAR VA at the visit compared with baseline, CRR, CRS, CRB, CRW, order by center, patient identification and date of visit.

13 Analysis of safety and tolerability

In CRS-1 and CRS-2 no safety information was collected.

The safety data of LEROS, as available, will be analysed using the Safety population.

13.1 Extent of exposure

The following information will be summarized from the Safety population:

- Total exposure to study treatment, expressed as person years (sum of duration of exposure to study treatment over all patients in days divided by 365.25)
- Average exposure: duration of exposure in days summarized using the number of observations (n), mean, standard deviation (SD), median, 1st and 3rd quartile, minimum and maximum
- Cumulative overall exposure: cumulative number and proportion of patients treated as tabulated in Table 13-1.

Table 13-1 – Extent of exposure: cumulative number and proportion of patients in the Safety population

	1 day	> 3 months	> 6 months	> 9 months	> 12 months	> 18 months	> 24 months
N	Xx	xx	xx	Xx	Xx	xx	xx
%	xx.x	xx.x	xx.x	xx.x	xx.x	xx.x	xx.x

Duration of exposure (days) is defined as: date of last dose of study medication – date of first dose of study medication +1.

13.2 Compliance

Treatment compliance will be calculated as percentage by dividing the number of tablets taken (tablets dispensed less tablets returned) by the number of days since the Raxone/idebenone first intake to Raxone/idebenone last intake multiplied by six and divided by 100.

For patients being compliant the calculated percentage must lie within the range of 80-120% (inclusively).

The number and proportion of patients who are compliant or non-compliant to the study treatment, as well treatment overall compliance as described in Table 13-2. In addition, the total number and proportion of patients who were non-compliant at least once during the study will be tabulated.

Table 13-2 LEROS medication compliance

Compliance as derived from eCRF	
Overall compliance, N mean (SD ; SE) median (Q_1 – Q_3) min-max	xx xx.xx (xx.xx ; xx.xx) xx.xx (xx.xx – xx.xx) xx.xx – xx.xx
Visit cumulative overall exposure, n(%)	
Yes (compliance 80%-120%)	xx (xx.x%)
No (compliance < 80%)	xx (xx.x%)

Listings: It be provided a listing with date of visit, visit type (baseline, follow-up month or unscheduled), start date of treatment, stop date of treatment, number of tablets dispensed, number of tablets returned, compliance, order by center, patient identification and date of visit.

13.3 Adverse events

In CRS-1 and CRS-2 no adverse events information was collected.

All adverse events (AEs) of patients participating in LEROS will be coded using most recent MedDRA version.

The treatment-emergent AEs (i.e., events which start or worsen during the study treatment) will be tabulated by system organ class (SOC) and preferred term (PT). Both subject and event counts will be calculated as described in Table 13-3.

Table 13-3 – AE by SOC and PT

	Events	**Patients**	**Rate**	**Days in Treatment[1]**	
				mean (SD)	**min – max**
Total patients with AE	xx	xx	xx.x	xx.x (xx.x)	xx.x – xx.x
SOC - PT	xx (xx.x%) xx (xx.x%)	xx (xx.x%) xx (xx.x%)	xx.x xx.x	xx.x (xx.x) xx.x (xx.x)	xx.x – xx.x xx.x – xx.x

[1] Days since start of treatment at the time of event

In addition, the treatment-emergent AEs will be evaluated by severity and by relationship to the study treatment. The serious AEs and AEs leading to a premature discontinuation will also be summarized as described in Table 13-4.

Table 13-4 - AE

	Events	Patients	Rate	Days in Treatment[1]	
				mean (SD)	min – max
AE	Xx	xx	xx.x	xx.x (xx.x)	xx.x – xx.x
Mild	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Moderate	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Severe	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
SAE	xx	xx	xx.x	xx.x (xx.x)	xx.x – xx.x
AESI	xx	xx	xx.x	xx.x (xx.x)	xx.x – xx.x

[1] Days since start of treatment at the time of event

TEAEs are defined as all AEs that start after the patient receives the first dose of study treatment.

The incidence (number and % of patients), frequency (number of events) and rate (incidence/frequency) following summaries will be provided:

- TEAE as displayed in Table 13-5.
- TEAEs broken down by PT, sorted in descending order of PT.
- TEAEs broken down by SOC and PT.
- TEAEs broken down by SOC, PT and severity. This summary will be done for the event count only and % will be calculated out of the total number of TEAEs.
- TEAEs broken down by SOC, PT and relationship to study medication. This summary will be done for the event count only and % will be calculated out of the total number of TEAEs.
- TEAEs leading to death broken down by SOC and PT.
- TEAEs other than those leading to death broken down by SOC and PT.
- TEAEs leading to permanent discontinuation broken down by SOC and PT.
- TEAEs leading to temporary drug interruption broken down by SOC and PT.

Table 13-5 - TEAE

	Events	Patients	Rate	Days in Treatment[1]	
				mean (SD)	min – max
TEAE	xx	xx	xx.x	xx.x (xx.x)	xx.x – xx.x
Drug-related	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Severe[2]	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Serious[2]	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
leading to permanent study treatment discontinuation	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
leading to temporary dose interruption	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
leading to death	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x

[1] Days since start of treatment at the time of event; [2] Other than death

Unless stated otherwise, the tables will be sorted by alphabetical order of the SOCs and then, within each SOC, by overall descending frequency of PTs based on the patient count. If only event count is presented, the sorting will be done based on the event count.

13.4 Laboratory safety variables

In CRS-1 and CRS-2 no laboratory data was collected.

For LEROS data, the clinical out of normal range laboratory variables with clinically significant values will be tabulated as described in Table 13-6. Clinical significance of the out of normal range laboratory variables will be as assessed by the investigator.

Table 13-6- Increased laboratory values

	Clinically Significant Events	Patients	Rate	Days in Treatment[1]	
				mean (SD)	min – max
Alanine Aminotransferase (ALT)	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Albumin	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x

Alkaline Phosphatase (ALP)	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Aspartate Aminotransferase (AST)	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Bicarbonate	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Calcium	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Chloride	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Cholesterol	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Creatine	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Gamma GT (GGT)	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Glucose	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Inorganic Phosphate	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Potassium	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Sodium	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Total Bilirubin	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Total Protein	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Triglycerides	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Urea or BUN	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Uric Acid	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Basophils	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Eosinophils	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
HGB	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Haematocrit	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Lymphocytes	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
MCH	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
MCHC	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x

MCV	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Monocytes	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Neutrophils	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Platelet Count	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
RBC Count	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
WBC Count	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Urine Blood	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Urine Glucose	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Urine Ketones	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Urine Protein	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x
Urine pH	xx (xx.x%)	xx (xx.x%)	xx.x	xx.x (xx.x)	xx.x – xx.x

[1] Days since start of treatment at the time of event;

Listings: It will be provided a listing with date of visit, visit type (baseline, follow-up month or unscheduled), lab test name, lab test value, lab test unit, order by center, patient identification and date of visit.

13.5 Vital signs

In CRS-1 and CRS-2 no vital signs information was collected.

Vital sign measurements include heart rate (HR), systolic and diastolic blood pressure (BP), respiratory rate. Actual values and change from baseline for each parameter at baseline and per follow-up visit will be summarized with descriptive statistics.

Additionally, potentially clinically significant vital signs will be identified for selected parameters as defined below. These abnormalities will be summarized using shift tables at baseline and per follow-up visit.

- Systolic BP: <90 mmHg, normal, >140 mmHg – 160 mmHg, >160 mmHg
- Diastolic BP: <50 mmHg, normal, >90 mmHg – 100 mmHg, >100 mmHg
- HR: <60 beats per minute (bpm), normal, >100 bpm.

Listings: It will be provided a listing with date of visit, visit type (baseline, follow-up month or unscheduled), supine systolic blood pressure (mmHg), supine diastolic blood pressure (mmHg), supine heart rate (beats per minute) and supine respiratory rate (breaths per minute), order by center, patient identification and date of visit.

13.6 Physical examination

In CRS-1 and CRS-2 no physical examination information was collected.

Listings: It will be provided a listing with date of visit, visit type (baseline, follow-up month or unscheduled), height and weight order by center, patient identification and date of visit.

13.7 Fundoscopic examination

In CRS-1 and CRS-2 no funduscopic examination information was collected.

The funduscopic examinations will be classified as Normal, Abnormal NCS or Abnormal CS for Examination findings. Data will be summarized by visit using shift tables.

Listings: It will be provided a listing with date of visit, visit type (baseline, follow-up month or unscheduled), left eye fundoscopic exam normal/abnormal, left eye examination findings, right eye fundoscopic exam normal/abnormal and right eye examination findings, order by center, patient identification and date of visit.

13.8 Other safety variables

No other safety data are to be analysed.

14 Deviations from the analyses planned in the current study protocol

There are currently no deviations from the CRS-1 CSP (version 1), CRS-2 CSP (version 1.0) and SNT-IV-005 CSP version 3.0 dated 06 March 2019 and SNT-IV-005 CSP addendum COVID-19 version1.0 dated 29 May 2020

15 Data analysis procedures

Statistical data analysis will be performed by 4Pharma Ltd according to current SAP.

Statistical analysis, tables and patient data listings will be performed with SAS® version 9.4 or higher for Windows (SAS Institute Inc., Cary, NC, USA).

16 Definitions

2nd eye onset

Most recent affected eye at Baseline.

Baseline (BL)

LEROS BL

NH BL

Best Visual Acuity

VA of the eye with best logMAR at the visit. However, if both eyes are off-chart, regardless of the VA, both eyes are considered as best eye.

Note: best eye at BL may not be the same as best eye at 6, 18 and 24 months or last assessment.

Clinical Relevant Benefit (CRB)

Defined as the composite of CRR and CRS, i.e, if the eye/patient has a CRR and/or a CRS then the eye/patient has also a CRB.

Clinical Relevant Recovery (CRR)

CRR is defined as improvement

- from "off-chart" (the equivalent of CF, HM, LP or NLP) VA to at least 1.6 logMAR value

or

- of at least 0.2 logMAR value within "on-chart".

Clinical Relevant Stabilization (CRS)

Is defined as maintenance of VA <1.0 logMAR in eyes with VA <1.0 logMAR at BL.

Clinical Relevant Worsening (CRW)

For any "on-chart" VA, CRW is defined as the disability to read at least 10 additional letters (equivalent to two chart lines) or a worsening to off-chart values (the equivalent of CF, HM, LP or NLP).

Delta onset

Is the time between symptoms onset of both eyes: date 2nd symptoms onset – date 1st symptoms onset. Patients will not be considered for calculating Delta onset if only one eye is affected or both eyes have the same date of symptom onset.

Months in Treatment

Months in treatment is only applied to LEROS patients. It is defined as time since BL to last follow-up during treatment period.

Nadir

Is the defined when VA reaches its worst point (highest logMAR or off-chart value). Time of nadir is the first time that nadir is reached.

Off-Chart

It is considered off-chart any VA entered as

- CF, HM, LP or NLP
- Any value above 1.68 logMAR

Patients ≤1 year after the onset of symptoms

A patient is considered ≤1 year after the onset of symptoms if, at BL, the most recent affected eye is ≤1 year after the onset of symptoms at BL for that eye.

Patients >1 year after the onset of symptoms

A patient is considered >1 year after the onset of symptoms if, at BL, the most recent affected eye is >1 year after the onset of symptoms at BL for that eye.

TEAE (Treatment Emergent Adverse Events)

TEAE that starts after the patient receives the first dose of study treatment.

Time in treatment at CRR by follow-up

Time in treatment at follow-up is divided in 5 categories:

- Before 6 months follow-up
- After 6 months follow-up up to 12 months follow-up
- After 12 months follow-up up to 18 months follow-up
- After 18 months follow-up up to 24 months follow-up

Visual Acuity blindness categories

VA categories are defined as follow:

- Non-legally blind: logMAR < 1.0;
- Legally blind: 1.0 ≤ logMAR ≤1.68
- Off-chart: CF, HM, LP or NLP

17 Data derivations and imputation rules

17.1 LEROS

Date of Birth:

- If month missing then 01JUL
- If Day missing (always) then 1st day of month

Onset date

- Day missing: 1st of the month
- Month missing: 1st of July

Idebenone start/stop, AE onset/stop date:

All dates are complete

Concomitant medication / Medical History start date:

Day missing: 1st of month

Month missing: January

Year missing: Set null

Concomitant medication /Medical History end date:

Day missing: last day of month

Month missing: December

Year missing: Set null

17.2 Natural History

17.2.1 Dates imputations

Idebenone start date

- Day missing
    - if there is a VA visit in the same month, then date of the visit
    - otherwise, 1st day of the month
- Month missing: 01 January
- Year missing: 01 January 1900

Idebenone stop date

- Day missing: last day of the month (28 for February)
- Month missing: 31 December
- Year missing; null

Onset date

- Day missing: 15th of the month
- Month missing: 1st of July

Visit date

- Day missing: 15th day of the month
- Month missing: 01 July
- Year missing: null

17.2.2 Visual Acuity Derivation

For visual acuity in CRS-1 it should be used the variable *logmar*.

CRS-2 logMAR values may not be available, therefore, a conversion it is necessary.

Conversion from decimal to logMAR: -log10(decimal)

Snellen to decimal: VASSNEL1 / VASSNEL2

Off-chart values to logMAR:

- CF: 2 logMAR
- HM: 2.3 logMAR
- LP: 2.6 loMAR
- NLP: 2.9 logMAR

18 References

European Medicines Agency: Guideline on Data Monitoring Committees. London 2007; EMEA/CHMP/EWP/5872/

Gao F, Thompson P, Xiong C, Philip Miller J. Analyzing Multivariate Longitudinal Data Using SAS. In: Proceedings of the Thirty-First Annual SAS Users Group International Conference, 187–31. Cary: SAS Institute, Inc. Paper 273-28 (2006).

19 Appendices

19.1 Matching algorithm workflow

Figure 19.1.1- LEROS eyes selection algorithm

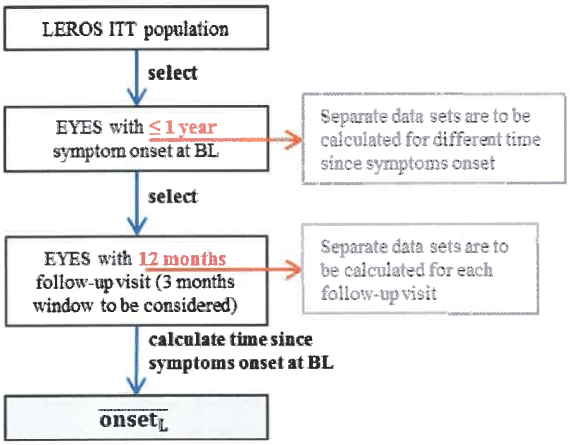
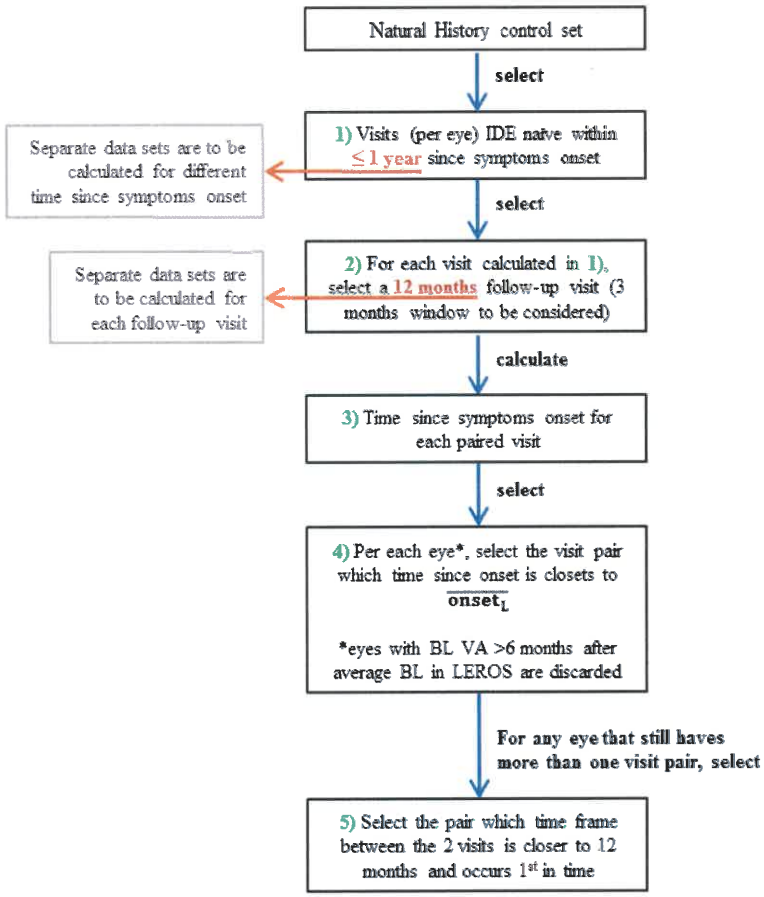
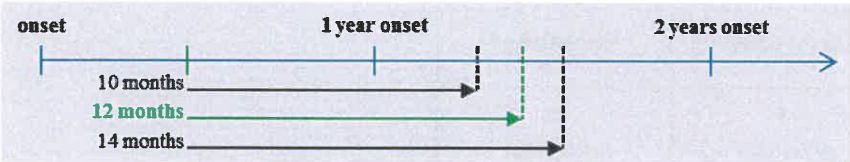


Figure 19.1.2 - Natural History eyes selection algorithm

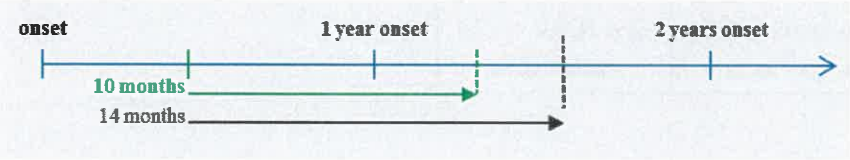


19.1.1 Natural History matching control pair selection example

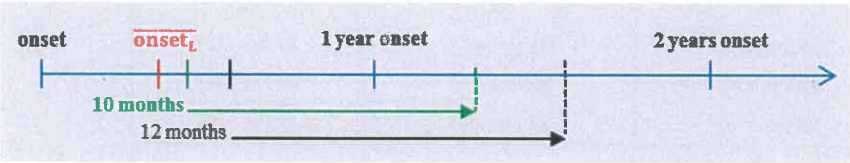
Natural History subject A



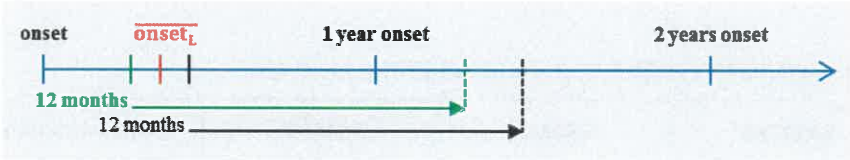
Natural History subject B



Natural History subject C



Natural History subject D



19.2 Table shell structure

Table 19-1 - Table shell for the comparison between LEROS and NH control group

Table Description	LEROS matched ITT	NH matched
Categorical variable, N	xx	xx
Category 1 n (%)	xx (xx.x%)	xx (xx.x%)
Category 2 n (%)	xx (xx.x%)	xx (xx.x%)
...	xx (xx.x%)	xx (xx.x%)
Continuos variable, N	xx	xx
mean (SD ; SE)	xx.xx (xx.xx ; xx.xx)	xx.xx (xx.xx ; xx.xx)
median ($Q_1 - Q_3$)	xx.xx (xx.xx – xx.xx)	xx.xx (xx.xx – xx.xx)
min-max	xx.xx – xx.xx	xx.xx – xx.xx

Table 19-2 - Table shell for LEROS ITT population

Table Description	2nd eye onset ≤ 1 year	2nd eye onset > 1 year	LEROS ITT
Categorical variable, N	xx	xx	xx
Category 1 n (%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
Category 2 n (%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
...	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
Continuos variable, N	xx	xx	xx
mean (SD ; SE)	xx.xx (xx.xx ; xx.xx)	xx.xx (xx.xx ; xx.xx)	xx.xx (xx.xx ; xx.xx)
median ($Q_1 - Q_3$)	xx.xx (xx.xx – xx.xx)	xx.xx (xx.xx – xx.xx)	xx.xx (xx.xx – xx.xx)
min-max	xx.xx – xx.xx	xx.xx – xx.xx	xx.xx – xx.xx

Table 19-3 - Table shell for primary LHON mtDNA mutations subgroup analysis

Table Description	G11778A	G3460A	T14484C	Other Mutation
Categorical variable, N	xx	xx	xx	xx
Category 1 n (%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
Category 2 n (%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
...	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
Continuos variable, N	xx	xx	xx	xx
mean (SD ; SE)	xx.xx (xx.xx ; xx.xx)	xx.xx (xx.xx ; xx.xx)	xx.xx (xx.xx ; xx.xx)	xx.xx (xx.xx ; xx.xx)
median ($Q_1 - Q_3$)	xx.xx (xx.xx – xx.xx)	xx.xx (xx.xx – xx.xx)	xx.xx (xx.xx – xx.xx)	xx.xx (xx.xx – xx.xx)
min-max	xx.xx – xx.xx	xx.xx – xx.xx	xx.xx – xx.xx	xx.xx – xx.xx

Table 19-4 - Table shell for age at baseline subgroup analysis

Table Description	< 18 years	≥ 18 and < 35 years	≥ 35 years
Categorical variable, N	xx	xx	xx
Category 1 n (%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
Category 2 n (%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
...	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
Continuous variable, N	xx	xx	xx
mean (SD ; SE)	xx.xx (xx.xx ; xx.xx)	xx.xx (xx.xx ; xx.xx)	xx.xx (xx.xx ; xx.xx)
median (Q_1 – Q_3)	xx.xx (xx.xx – xx.xx)	xx.xx (xx.xx – xx.xx)	xx.xx (xx.xx – xx.xx)
min-max	xx.xx – xx.xx	xx.xx – xx.xx	xx.xx – xx.xx

Table 19-5 - Table shell for Blindness Category of Best VA at BL subgroup analysis

Table Description	Off-chart	Legally blind	Non-legally blind
Categorical variable, N	xx	xx	xx
Category 1 n (%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
Category 2 n (%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
...	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
Continuos variable, N	xx	xx	xx
mean (SD ; SE)	xx.xx (xx.xx ; xx.xx)	xx.xx (xx.xx ; xx.xx)	xx.xx (xx.xx ; xx.xx)
median (Q_1 – Q_3)	xx.xx (xx.xx – xx.xx)	xx.xx (xx.xx – xx.xx)	xx.xx (xx.xx – xx.xx)
min-max	xx.xx – xx.xx	xx.xx – xx.xx	xx.xx – xx.xx

Table 19-6 - Table shell for Safety population

Table Description	Safety Population
Categorical variable, N	xx
Category 1 n (%)	xx (xx.x%)
Category 2 n (%)	xx (xx.x%)
...	xx (xx.x%)
Continuos variable, N	xx
mean (SD ; SE)	xx.xx (xx.xx ; xx.xx)
median (Q_1 – Q_3)	xx.xx (xx.xx – xx.xx)
min-max	xx.xx – xx.xx

Table 19-7 - Table shell for Survival Analysis using Kaplan-Meier method

Variable in analysis	6 months	12 months	18 months	24 months	Last Visit*
ITT patients					
At risk, n (%)[1]	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
Event, n (%)[1]	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
Censored, n (%)[1]	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
KM, [95%CI] [2]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]
RKM, [95%CI] [3]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]
SE[4]	xx.x	xx.x	xx.x	xx.x	xx.x
Subgroup 1					
At risk, n (%)[1]	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
Event, n (%)[1]	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
Censored, n (%)[1]	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
KM, [95%CI] [2]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]
RKM, [95%CI] [3]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]
SE[4]	xx.x	xx.x	xx.x	xx.x	xx.x
Subgroup ...					
At risk, n (%)[1]	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
Event, n (%)[1]	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
Censored, n (%)[1]	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
KM, [95%CI] [2]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]
RKM, [95%CI] [3]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]	xx.x% [xx.x,xx.x]
SE[4]	xx.x	xx.x	xx.x	xx.x	xx.x

[1] **n (%)** values are presented as frequency and percentages; [2] **KM:** Kaplan-Meier estimate; [3] **RKM:** Reverse Kaplan-Meier estimate; [4] **SE**: Standard Error