Supplemental Data: A

CLINICAL SITES

A total of 111 patients had been enrolled by treating physicians at 38 different sites in 10 countries. Most enrolling sites were in Europe (N=27), followed by the US (N=6) and Australia/New Zealand (N=5).

DATA

Data collection

Data was collected from available medical records, by means of Case Record Forms (CRF):

- Demographic data (age, gender)
- Date of onset of symptoms on each eye
- Genetic confirmation (mutation)
- Best Corrected Visual Acuity (BCVA) (see below)
    - At start of treatment (Baseline)
    - At follow-up visits
- Date of each visit
- Dose
- Adverse events

Statistical Methods

There was no planned sample size as all requests for access to idebenone for eligible patients which were bona fide and unsolicited had been granted. All treating physicians were approached and invited to contribute data from their treated patients.

Efficacy criteria was based in the Responder Analyses (CRR, CRS and CRB) (see below) with Best Corrected Visual Acuity (BCVA) as efficacy variable. BCVA was assessed using ETDRS (Early Treatment Diabetic Retinopathy Study) charts with logMAR (logarithm of the minimal angle of resolution) values as units. In cases where VA was assessed using Snellen fraction/units, logMAR values where calculated using standard conversion methods.

If VA was > 1.68 logMAR or off-chart (regardless of being assessed as counting fingers, hand motion, light perception or no-light perception) it was imputed to 1.8 logMAR in order to standardize visual acuity data from different physicians. The value 1.8 logMAR was based on the CRR definition: it is considered a CRR any off-chart VA that recovers to at least 1.6 logMAR (being 1.6 logMAR the equivalent to reading one full line in the ETDRS chart).

Continuous data was summarised using the mean, standard deviation, median, 1st and 3rd quartiles, minimum and maximum. Categorical data was presented in contingency tables with frequencies and percentages.

CRR was summarised by means of descriptive statistics and Kaplan-Meier estimates, presented with the 95% confidence interval (using the Greenwood formula) and reverse Kaplan-Meier curves. Unless stated otherwise data was analysed using the observed cases or missing data were imputed with the last available observation carried forward (LOCF).

RATIONALE FOR EFFICACY OUTCOMES (CLINICALLY RELEVANT RECOVERY, CRR, AND CLINICALLY RELEVANT STABILIZATION, CRS)

In line with the approach previously used in RHODOS, efficacy can be evaluated both in terms of improvement as well as maintenance of VA. In a rapid and severely progressive disease for which the most frequent outcome is a disabling degree of blindness, recovery of visual function is a desirable outcome. Likewise, prevention of VA deterioration is also a therapeutic objective, especially if achieved when the degree of visual dysfunction is still small and patient's autonomy is preserved.

On the other hand, in order to avoid VA variability interfering with efficacy analysis and potentially driving clinically unmeaningful statistical significance, a responder approach was chosen for determining efficacy both in terms of recovery (CRR) and prevention of deterioration or stabilization (CRS).

CRR

In order to ensure that improvement in VA is clinically meaningful and "recovery" within "off-chart" VA categories is not over-emphasized, a Responder Analysis was employed for the assessment of efficacy of idebenone, in which only patients presenting with a clinically relevant degree of VA recovery would be defined as "responders". This approach reduces the potential for over-emphasizing confounding influences of clinically less important VA changes (e.g. changes between "off-chart" VA categories) and of day-to-day variability in VA. This clinically relevant recovery (CRR) had been reported in the literature[8] and was also used in the post-hoc analysis of the RHODOS trial, ~~as well~~ and as an efficacy outcome parameter in the study LEROS (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) (ongoing at the date of submission)). It was also proposed by an international consensus on the management of LHON[4]. The criteria for the classification of a responder are described below (see Definitions, CRR).

CRS

CRS evaluates the capacity of therapy to prevent deterioration into a more severe category of visual impairment, without considering the numerical magnitude of VA change intra-category. In patients starting therapy when VA is still near normal or moderately impaired and, in the context of a rapidly progressing pathology, prevention of further deterioration to "legal blindness" is important for patient's autonomy. This approach was also employed in RHODOS.

PATIENT DISPOSITION/ANALYSIS POPULATIONS

Data from a total of 111 patients was collected. The following populations were defined for the analysis of safety and efficacy data:

- Safety Population (SP): used for analysis of safety information. It includes all patients enrolled in the EAP who received at least one dose of idebenone (111 patients).
- Efficacy Population (EP): is defined as the sub-population of the SP who carried one of the 3 major LHON-causative mtDNA mutations, who had time since onset at Baseline of less than 12 months in the most recently affected eye and for whom post-Baseline VA efficacy data was available (87 patients).

DEFINITIONS

- **Nadir**: Nadir is defined as the value when VA reaches its worst point (highest logMAR value). Time of nadir is the first time that nadir is reached, which can take place at baseline, or during the course of the treatment.

- **CRR (Clinically Relevant Recovery)**: It is defined as an 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"

(cf. Supplemental Figure 1)

As response criteria, **all eyes/patients** qualified as responders (that is, that have CRR) are only considered so when the criteria is evident at the **last available observation (last visit)**. If a patient/eye shows a CRR at a visit during follow-up, but not at the last visit, **it is not considered as a responder**, and no CRR is accounted for.

CRR can be observed when last observation is compared to the *baseline* value (*CRR from Baseline*) or when last observation is compared to the *nadir* (*CRR from Nadir*).

When evaluating CRR in **patients** it is considered that:

- a patient has a CRR if at least one eye has a CRR;
- time of CRR is the time when the 1st CRR occurred;
- improvement of VA at CRR is the improvement observed at the time of 1st CRR;
- improvement of VA at last visit is the best improvement observed in both eyes.

- **CRS (Clinically Relevant Stabilisation of residual VA)**: is defined as a patient having a logMAR of <1.0 at Baseline (below the threshold of severe vision loss, legal blindness in the United States) in at least one eye and maintaining a logMAR of <1.0 in that eye at their last follow-up assessment. A patient has a CRS if at least one eye has a CRS.

- **Magnitude of Improvement**: "Magnitude of improvement from baseline" is defined as the difference between VA logMAR at the visit and VA logMAR at baseline. "Magnitude of improvement from nadir" is defined as the difference between VA logMAR at the visit and VA logMAR at nadir.
  - A decrease in logMAR of 0.02 (-0.02) is equivalent to an improvement in reading ability of one letter (+1 letter) and an increase in logMAR of 0.02 (+0.02) is equivalent to the deterioration in reading ability of one letter (-1 letter).

- **Visual Impairment Categories**: Both at eye and subject level, BCVA values (in logMAR) were classified in three categories (This classification allows to observe changes related to quality of life relevant to the patient's function.)

  (cf. Supplemental Figure 2)

  - **Off-chart:** not reading any letter on the ETDRS chart at 1m (i.e. >1.68 logMAR)
  - **From 1.0 to 1.68 logMAR:** not reading any letter on the ETDRS chart at 4m (i.e. >1.00 logMAR) but being able to read at least one letter on the ETDRS chart at 1m (i.e. 1.68 logMAR)
  - **<1.0 logMAR:** Being able to read at one or more letters on the ETDRS chart at 4m.