Full/Long Title Of The Study: Ravicti as therapy for bestrophinopathies: a double-blind crossover randomized controlled trial

Short Study Title/Acronym: Bestrophin 1 treatment trial on the effectiveness of Ravicti / BETTER study

Statistical analysis plan amendments:

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Signatures:



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Introduction

This is a double-blind crossover randomized control trial to examine the effectiveness of Ravicti in patients with clinical diagnosis and molecular confirmation of autosomal dominant best vitelliform macular dystrophy (BVMD) or autosomal recessive bestrophinopathy (ARB). Patients aged between 18 and 65 years, with their best corrected visual acuity recorded as better than hand movements at recruitment, who are able to speak and understand English and give consent will be randomised to two arms where half of the participants will take placebo in the first period and glycerol phenylbutyrate in the second, and the other half will take glycerol phenylbutyrate first and placebo second. Potential participants will be identified by the clinical care team at MFT using the database of The North West Genomic Laboratory Hub considering both patients under MFT and external individuals willing to travel to MFT for study purposes.

Clinical investigation timetable and termination

Recruitment is expected to take 6 months with 7 days for each treatment and a follow-up period of 21 days for each study phase, totalling 56 days.

Principal Research Aims

The study hypothesis is that treatment with glycerol phenylbutyrate will increase mutant bestrophin 1 activity in patients with bestrophinopathy. Thus, the primary objective is to demonstrate whether this treatment results in a significantly increased EOG LP:DT ratio, compared to treatment with a placebo, after 7 days of dosing.

The null hypothesis is that treatment with glycerol phenylbutyrate has no significant effect on the EOG compared to a placebo.

Secondary Research Aims

If there is a change in the EOG LP:DT ratio after dosing with glycerol phenylbutyrate, does it return to baseline 21 days after cessation of treatment?

Sample Size

The primary outcome is LP:DT ratio at Days 8 or 36. Data are available from an audit of Manchester Royal Eye Hospital patients (n=19) who were referred with a diagnosis of bestrophinopathy, and who had abnormal EOGs and normal ERGs. These patients had mean LP:DT ratio of 1.109 (SD 0.158) in the worst eye. Based on this, a sample size of 10 will allow us to detect an effect size of 1.00 with 80 % power and alpha of 0.05, using a paired analysis. This is inflated to 12 to allow for 10% dropout and

missing data. Although no data are available on the within-patient variance, this is expected to be less than the between-patient variance and therefore we will have the power to detect a difference of at least 0.16 (14.2% change).

Study Outcomes and Measures

Primary Outcome

EOG LP:DT ratio measured on Days 8 and 36.

Secondary Outcomes

EOG LP:DT ratio measured on Days 1 and 29 (baseline periods 1 and 2) and at Day 57.

Statistical Analysis Methods

All variables will be presented using appropriate descriptive statistics (mean/median, standard deviation [SD] / interquartile range [IQR], N and percentage) and graphics to examine for completeness and form. No formal imputation will be made for missing values, but, in the event of large numbers, sensitivity analyses may be utilised. All analyses will be intention to treat, with sensitivity analyses used in the event of protocol deviations.

To reduce potential sources of bias, all EOG measurements will be taken at the same time each day morning or afternoon, by a member of the clinical electrodiagnostic staff. In the event of any deviations from protocol, they will be reported and sensitivity analyses may be used to exclude that individual. Patients will be randomised to either sequence A (glycerol phenylbutyrate then protocol) or sequence B (Protocol then glycerol phenylbutyrate). The statistician will be blinded to the treatment.

Calculation of EOG

The normal way of analysing EOG is to find the minimum during the dark phase and compare this with the maximum in the light phase, however with this population there are problems.

Firstly, all the data are very low compared with normal. There is often a small step between the last dark-adapted run and the first light-adapted, which generally gets lost, however for some participants it creates an artificially high Arden Index even though there is absolutely no change in signal over the light phase. Previous discussions suggested as our baseline, the first runs in the light phase, rather than the lowest runs in the dark phase, potentially using an average of the first three, but this was also problematical. For instance, the first light run is always noisy because it's difficult to keep your eyes open. Therefore, the value from the second light run will be taken as the baseline with no

averaging. In the event of participants with further issues alternative methods will be investigated as a sensitivity analysis.

Outlier Identification

Values will be taken to be an outlier if they exceed the average of the single surrounding points by more than defined margin (C). This is scaled from 0 to 99. For each observation within an individual and timepoint we compute the ratio, R, of value n to the mean of values n-1 and n+2 (thus this would be 1.0 if they are invariant). R is then accepted if between 1+ C and 1 – C, otherwise reject. For the first iteration of analyses C will be set as 0.3 (30%). Subsequently all data will be reviewed for sense, with alternative values of C applied on an individual basis and any further manually identified outliers will be removed in sensitivity analyses.

Summary of baseline data and flow of patients

Due to the small sample size any adjustment for confounders will be considered only in the event of a large number of deviations on a single factor.

Baseline EOG will be obtained on Days 1 and 29. These will be descriptively compared using summary statistics and boxplots. All baseline data will be reported overall and for the two randomisation arms.

Primary outcome analysis

For the primary analysis, outcomes on Days 8 (period 1) and 36 (period 2) will be reported descriptively by treatment. Baseline EOG will be obtained for each period on Days 1 and 29 respectively. Comparisons will be made between baselines for period 1 and Day 8, and for period 2 and Day 36, using a paired t-test. Glycerol phenylbutyrate and placebo will be compared using a linear mixed model with period, treatment and the within-subject difference in baseline responses as fixed effects and subject as a random effect (21). Each subject will serve as their own control in the crossover study design and the model will account for correlated data from the same subject. A 95% confidence interval will be reported for the difference between glycerol phenylbutyrate and placebo based upon the linear mixed model.

Secondary outcome analysis

Secondary analyses will explore the trajectory over time from baseline to Day 57. This will be presented via a line graph and reported with descriptive statistics. As the secondary hypothesis is of no change from baseline, any tests will follow the primary analysis methods and will be exploratory.

Exploratory comparisons will be made between the baseline from both Days 1 and 29 and the Day 57 EOG using descriptive statistics and graphics.

Subgroup analyses

Descriptive analyses will present the results for individuals subdivided into BVMD and ARB. Due to the small numbers no formal statistical tests will be performed.

Adjusted analysis

This is a crossover design where all individuals will act as their own control. No adjustment will be made for any differences between the two randomised groups The use of a linear mixed effects model will incorporate adjustment for the baseline EOG from the start of each period.

Interim analysis and criteria for the premature termination of the trial

No interim analysis will take place. The study sponsor and the investigator reserve the right to terminate the study. The investigator or the national study sponsor, if applicable, will notify the REC/MHRA in writing of the study's completion or early termination. These criteria may include safety issues, noncompliance, significant concerns (e.g., complaints by a participant or any other party), or an inspection finding(s).

Participant population

The population will be all-randomised, i.e. any participant randomised into the trial, regardless of whether they received the trial drug, will be included in the analysis.

All analyses will be performed as intention to treat. All randomised individuals with available relevant data will be included in the analyses regardless of whether they received treatment as per protocol.

Procedure(s) to account for missing or spurious data

No formal imputation will be made for missing values, but, in the event of large numbers, sensitivity analyses may be utilised with post-hoc imputation, for example the use of the other baseline in the event of missing data at Days 1 or 29.

Dummy tables

Table 1 (for construction of): CONSORT flow diagram

Screening variable	Participant numbers
Participants assessed	
Included	
Excluded	
Not meeting inclusion	
Declined to participate	
Other reasons	
Consented	
Lost to follow-up (reason)	
Analysed	

Table 2: Participant descriptive statistics (baseline)

Participant variable at baseline	Full cohort
Age, years; mean (SD)	
Male gender, n (%)	
Height, cm; mean (SD)	
Weight, kg; mean (SD)	
Visual acuity OD, logMar; mean (SD)	
Visual acuity OD, M; mean (SD)	
Visual acuity OS, 1logMar; mean (SD)	
Visual acuity OS, M; mean (SD)	

Table 3: Primary Outcome - EOG

Timepoint	Group 1	Group 2
Baseline		
Day 1		
Day 8		
Day 29		
Day 36		
Day 57		

Table 4: Other Outcomes

Timepoint	ERG		FAF		OCT	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Baseline						
Day 1						
Day 8						
Day 29						
Day 36						
Day 57						

Table 5: Adverse Events

Adverse Event n (%)	Full cohort