

FULL/LONG TITLE OF THE TRIAL

Ravicti as therapy for bestrophinopathies: a double-blind crossover randomized controlled trial

SHORT TRIAL TITLE/ACRONYM

Bestrophin 1 treatment trial on the effectiveness of Ravicti / BETTER study

Sponsor reference number: B01914

Sponsor: Manchester University NHS Foundation Trust

Funder: MRC

Funder reference: MR/W021633/1

IRAS ID: 1006836

Protocol version: 1.6

Protocol date: 2nd May 2025

This protocol has regard for the HRA guidance and order of content

This project will be conducted in accordance with the study protocol and the ethical principles outlined by Good Clinical Practice (GCP) and the Declaration of Helsinki in its most current version.



RESEARCH REFERENCE NUMBERS

IRAS Number: 1006836
ISRCTN Number: ISRCTN15509883

SPONSORS Number: B01914
FUNDERS Number: MR/W021633/1



SIGNATURE PAGE

The sponsor signature on the IRAS form, acts as documented acceptance that the sponsor approves the protocol.

The Chief Investigator should sign below to confirm the following:

The Chief Investigator confirms the protocol has been agreed and accepted and agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the sponsor's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the sponsor.

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

Chief Investigator:

Signature:

.....

Date:

...../...../.....

Name: (please print): Dr Eva Lenassi

.....

(Optional)

Statistician:

Signature:

.....

Name: (please print): Dr Catherine Fullwood

.....

Position:

.....



KEY TRIAL CONTACTS

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Funder	DPFS Award: Medical Research Council Developmental Pathway Funding Scheme award for 'BESTrophin 1 Treatment Trial on the Effectiveness of Ravicti (BETTER)' starting February 2023. £194,144. Supplement to DPFS Award: £5,691
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Committees	Trial Management Group (TMG)- contact trial co-ordinator Trial Steering Committee (TSC)
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ii. LIST OF ABBREVIATIONS

4PBA	Sodium phenylbutyrate
AE	Adverse Event
AR	Adverse Reaction
ARB	Autosomal Recessive Bestrophinopathy
ATF6	Activating Transcription Factor 6 (gene)
BEST1	Bestrophin 1 (gene)
BVMD	Best Vitelliform Macular Dystrophy
CI	Chief Investigator
Cl ⁻	Chloride ion
Co-I	Co-Investigator
CRF	Case Report Form
CTA	Clinical Trial Authorisation
CTIMP	Clinical Trial of Investigational Medicinal Product
CYP2D6	Cytochrome P450 2D6 (protein)
CYP3A4	Cytochrome P450 3A4 (protein)
DSUR	Development Safety Update Report
DT	Dark Trough
EMA	European Medicines Agency
EOG	Electro-oculogram
ERG	Electroretinogram
FAF	Fundus autofluorescence
FDA	United States Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HRA	Health Research Authority
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
IQR	Interquartile Range
ISF	Investigator Site File (This forms part of the TMF)



ISRCTN	International Standard Randomised Controlled Trials Number
LP	Light Peak
MFT	Manchester University NHS Foundation Trust
MHRA	Medicines and Healthcare products Regulatory Agency
MRC	Medical Research Council
MREH	Manchester Royal Eye Hospital
N	Number
NHS	National Health Service
OCT	Optical Coherence Tomography
PI	Principal Investigator
PAA	Phenylacetic Acid
PIC	Participant Identification Centre
PIS	Participant Information Sheet
PPI	Public and Patient Involvement
QP	Qualified Person
R&D	Research and Development
REC	Research Ethics Committee
RN	Research Nurse
RPE	Retinal Pigment Epithelium
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SD	Standard Deviation
sIMPD	Simplified IMPD
SLC6A1	Solute Carrier Family 6 Member 1 (protein)
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
STXBP1	Syntaxin-binding protein 1 (protein)
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee
UCD	Urea Cycle Disorder
VA	Visual Acuity



iii. TRIAL SUMMARY

Trial Title	BEstrophin 1 Treatment Trial on the Effectiveness of Ravicti (BETTER study)	
Internal ref. no. (or short title)	B01914	
Clinical Phase	2	
Trial Design	Double-blind crossover randomized control trial	
Trial Participants	Patients with clinical diagnosis and molecular confirmation of autosomal dominant best vitelliform macular dystrophy (BVMD) or autosomal recessive bestrophinopathy (ARB)	
Planned Sample Size	12	
Treatment duration	7 days (IMP) & 7 days (placebo)	
Follow up duration	21 days (-7/+14) IMP 21 days (-7/+14) Placebo	
Planned recruitment period	15 th May 2024 – 18 th June 2025	
Planned Trial Period (from Sponsor Green Light to end of follow up period)	15 th May 2024 – 17 th September 2025	
	Objectives	Outcome Measures
Primary	Does the electro-oculogram (EOG) light peak to dark trough (LP:DT) ratio increase after 7 days of glycerol phenylbutyrate dosing in patients with BVMD or ARB.	EOG LP:DT ratio at 7 days
Secondary	If there is a drug-related change in the EOG, does it return to baseline?	EOG LP:DT ratio at 21 (-7/+14) days post-cessation.
Investigational Medicinal Product(s)	Glycerol phenylbutyrate (Ravicti ®, Immedica)	
Formulation, Dose, Route of Administration	Colourless to pale yellow liquid, 1.1 g/mL of glycerol phenylbutyrate. 11.2 ml/m ² /day (12.4 g/m ² /day) body surface area given orally in 3 equally divided dosages rounded up to the nearest 0.5 ml for 7 days.	



iv. FUNDING AND SUPPORT IN KIND

FUNDER(S) (Names and contact details of ALL organisations providing funding and/or support in kind for this trial)	FINANCIAL AND NON FINANCIAL SUPPORT GIVEN
Medical Research Council (MRC)	£194,143.52 (initial award) £5,691.20 (budget supplement)

v. ROLE OF TRIAL SPONSOR AND FUNDER

Manchester University NHS Foundation Trust (MFT) is acting as sponsor for this study and is assuming overall responsibility for the initiation and management of the study. The Trust will provide permission to conduct the research and monitor the progress of that research. The research team all hold substantive or honorary contracts with the Trust and therefore the sponsor has influence over all aspects of the study design, conduct, data analysis and interpretation, manuscript writing, and dissemination of results which are the responsibility of the research team. The Funder’s role will be according to the contract to be signed between Funder and MFT.

vi. ROLES AND RESPONSIBILITIES OF TRIAL MANAGEMENT COMMITTEES/GROUPS & INDIVIDUALS

Trial Management Group (TMG): Members will be: Chief Investigator (CI, chair), Lead Sub-Investigator (co-chair), Sub-Investigators, Statistician, Clinical Trial Manager, Clinical Trial Pharmacist, Research Coordinator/Practitioner, Patient Representative and Sponsor representative. The TMG will meet every 2 months either face to face or remotely. TMG will be quorate if two Investigators including chair or co-chair, Clinical Trial Manager, pharmacist and Research Coordinator/Practitioner are present. TMG will monitor all aspects of the conduct and progress of the trial, ensure that the protocol is adhered to and take appropriate action to safeguard participants and the quality of the trial itself.

The TMG will:

- Assess evidence of participant harm (e.g. Serious Adverse Events (SAEs), deaths)
- Assess data quality
- Assess new or external evidence



- Recommend protocol amendments as proposed by sponsor or investigators
- Recommend that recruitment be continued or stopped
- Monitor compliance with previous recommendations

Trial Steering Committee (TSC): Chair (Professor Bernadette Brennan), an independent expert in bestrophinopathies, an independent statistician, the CI, the trial co-ordinator, and a sponsor representative (Ms Lynne Webster). The study statistician will attend every meeting. TSC will meet twice a year or ad hoc as needed. The TSC will send reports to the sponsor. The TSC will be independent of the sponsor and investigators. The TSC will undertake activities as part of their remit outlined in the TSC Charter.

vii. Protocol contributors

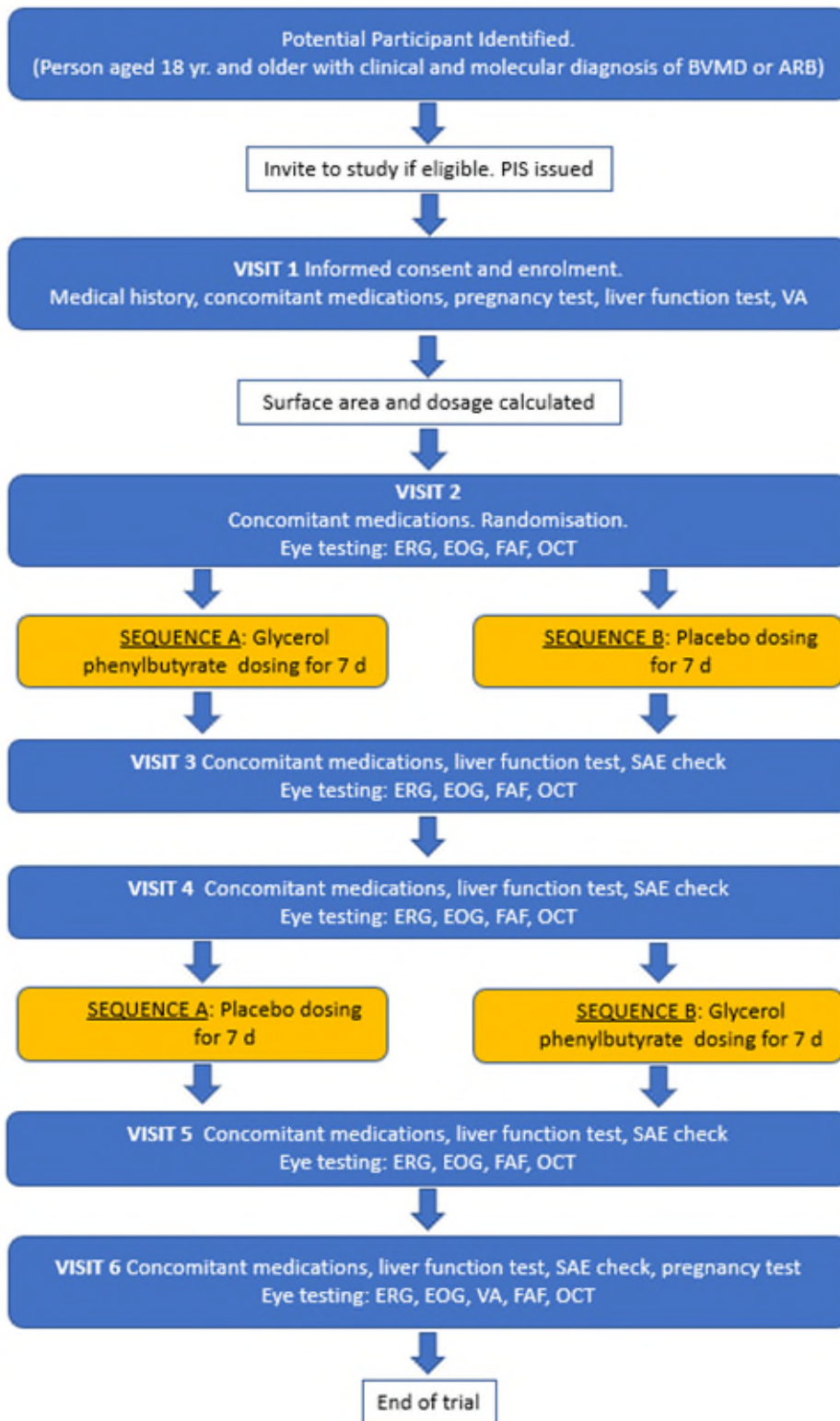
1. Dr Forbes Manson: Protocol development and design
2. Prof Neil Parry: Clinical science sections and protocol design
3. Dr Eva Lenassi: Protocol development and design
4. Dr Catherine Fullwood: Protocol statistical elements
5. Dr Miriam Lettieri: Pharmacy section
6. Sarah Bowers: Protocol Development and design

viii. KEY WORDS:

bestrophin 1, bestrophinopathy, ARB, BVMD, glycerol phenylbutyrate, Ravicti, electro-oculogram, electroretinogram, randomised.



ix. TRIAL FLOW CHART



1 BACKGROUND

This study will investigate whether phenylbutyrate (4PBA) in the form of glycerol phenylbutyrate is an effective treatment for bestrophinopathies, a group of untreatable inherited retinal disorders leading to sight loss. Bestrophinopathies include autosomal dominant BVMD and ARB and result from mutations in the *BEST1* gene. *BEST1* encodes the bestrophin 1 protein, a chloride (Cl⁻) channel in the pigmented layer of the retina, i.e. the retinal pigment epithelium (RPE). *BEST1* mutations result in absent or reduced Cl⁻ conductance, characteristically manifesting clinically as an abnormal EOG (1, 2). In this study the EOG will serve a proxy measure of bestrophin 1 function.

4PBA, and its pro-drug glycerol phenylbutyrate, are licenced for the treatment of urea cycle disorders (UCDs). 4PBA has a secondary action of increasing the function of mutant proteins. The mode of action is unknown but 4PBA may help mutant proteins fold correctly or induce the expression of other proteins that help mutant proteins attain their correct structure, so escaping degradation. 4PBA may also facilitate partially functional mutant proteins, that would normally be degraded, to be retained and sent to the correct cellular location (3, 4). The researchers have shown 4PBA fully restores Cl⁻ conductance to mutant ARB bestrophin 1 and partially restores function to mutant BVMD bestrophin 1 in cell models and patient RPE derived from stem cells (5, 6).

4PBA (Buphenyl[®]) has been approved for clinical use since 1996 for the treatment of UCDs. Ravicti[®] (glycerol phenylbutyrate) is a more acceptable formulation of 4PBA to patients because it is an odourless, tasteless liquid. Ravicti is approved for use in patients from the age of 2 months. Ravicti contains 3 molecules of 4PBA for every molecule of triglyceride. After oral ingestion, the 3 molecules of 4PBA are released from the glycerol backbone in the gastrointestinal tract by lipases.

A large number of cell, animal and human studies have shown the efficacy of 4PBA in restoring function to mutant proteins with missense mutations and rescuing the disease phenotype. In mice 4PBA was able to ameliorate cone photoreceptor survival and vision in a model of Leber congenital amaurosis. Furthermore, the topical application of 4PBA restored normal intraocular pressure in a transgenic myocilin mouse model of primary open angle glaucoma (7, 8). Oral administration of 4PBA to mice that were transgenic for mutant human α 1-antitrypsin resulted in blood levels of α 1-antitrypsin increasing to 20-50% of those seen in transgenic mice expressing wild type α 1-antitrypsin (9). A follow-up study in humans that undertook a 14-day oral course of 4PBA had no effect on α 1-antitrypsin blood levels (10). Treatment of cystic fibrosis patient primary cells with 4PBA restored forskolin-activated Cl⁻ secretion and the post-translational modification of Δ F508-CFTR (11). In a follow-up clinical trial, orally administered 4PBA improved the nasal potential difference response in cystic fibrosis patients with minimal side-effects (12, 13).



ClinicalTrials.gov lists 68 studies (43 completed, 5 recruiting) with buphenyl (including phenylbutyrates and phenylbutyric acid) and 21 studies (14 completed, 1 active, 1 recruiting, 1 planned) with Ravicti (including drug name glycerol phenylbutyrate and synonyms HPN-100 and GT4P). As well as UCDs the conditions under investigation include STXBP1 encephalopathy and SLC6A1 neurodevelopmental disorder, Allan-Herndon-Dudley syndrome, Wolfram syndrome, Amyotrophic Lateral Sclerosis, cystic fibrosis and a planned trial for ATF6 achromatopsia patients. Trials typically use, or plan to use, the drug at its maximum daily dose (typical Buphenyl doses for the treatment of UCDs is 400-600 mg/kg/day for patients up to 20 kg, or 9.9-13.0 g/m²/day for patients heavier than 20kg). In this trial, the maximum daily dose of Ravicti will be 18 ml (1.1 g/ml, or 19.8 g) (total daily dosage of Ravicti (ml) = total daily dosage of Buphenyl (sodium phenylbutyrate) tablets (g) x 0.86).

The most common side effects of Ravicti in adults include diarrhoea, flatulence, headache, abdominal pain, vomiting, tiredness, decreased appetite and indigestion or heartburn. Phenylacetate is a breakdown product of Ravicti and may cause nervous system side effects that include sleepiness, light-headedness, change in taste, problems with hearing, confusion, problems with memory, worsening of numbness, tingling or burning in hands or feet, headache, fatigue, nausea and vomiting.

2 RATIONALE

4PBA is well documented to increase the function of mutant missense proteins associated with several different conditions. The researchers have reported that 4PBA increases the function of mutant bestrophin 1 associated bestrophinopathies (ARB and BVMD) in cell models. For ARB the increased function equalled that of wild type bestrophin 1, and for BVMD there was a significant increase in mutant bestrophin 1 function.

Bestrophinopathies can lead to irreversible sight loss and there is presently no proven treatment for this group of conditions. People rank sight as their most important sense, and its loss has a significant detrimental impact on an individual's quality of life. This research will endeavour to 'ensure no one gets left behind just because they have a rare disease' (UK Strategy for Rare Diseases, 2013).

In this study the researchers will test whether 4PBA, in the form of glycerol phenylbutyrate (Ravicti), increases the function of mutant bestrophin 1 in patients with either ARB or BVMD. Bestrophinopathy patients have a characteristic abnormal EOG (1, 2). Here, patient EOG measurements will serve as a proxy for bestrophin 1 function.

The EOG is a measure of outer retina and RPE function. It is determined by measuring the standing potential between the front of the eye (cornea) and the Bruch's membrane (which is located under the



retina) (14). This measure differs under light and dark conditions. After about 15 minutes dark adaptation the standing potential reaches a minimum level (DT); a maximum is reached after a similar period in the light (LP). The LP results from increased free intracellular calcium released from the endoplasmic reticulum. Bestrophin 1 has an essential role in this. The increased intracellular calcium concentration opens channels in the basolateral membrane of the RPE, causing cells to depolarise. Thus, the measurement of the LP:DT ratio is an indirect measurement of bestrophin 1 activity. The LP:DT ratio (Arden ratio) is normally 1.80. In bestrophinopathies, the ratio is characteristically less than this, or absent (14).

In this study's double-blind cross-over design, half the subjects will take placebo in the first window and glycerol phenylbutyrate in the second, while the other half will take glycerol phenylbutyrate first and placebo second. The protocol has been designed to introduce randomisation. It will not be possible to use historical information borrowing as there are no prior studies which could be used.

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 for primary and secondary endpoints based upon a linear mixed model.

Participants will be dosed based on body surface area at 11.2 ml/m²/day (12.4 g/m²/day) given in three equally divided dosages rounded up to the nearest 0.5 ml for 7 days. The total daily dosage will not exceed 18 ml (19.8 g). This complies with the prescribing recommendations for patients naïve to 4PBA (15) and it is rounded to the nearest 0.5ml to make the withdrawal easier for the subject/carer. Seven days of glycerol phenylbutyrate dosing has been chosen as this will be sufficient time to allow for protein turnover and allow new bestrophin 1 to be translated in the presence of glycerol phenylbutyrate.

For two completed CTIMPs that tested whether 4PBA (Buphenyl) restored CFTR function in cystic fibrosis patients the doses ranged from 19-40 g/day for 7 days (11, 12). This is equivalent to 16.3-34.4 ml (18.0-37.8 g) Ravicti (15). The latest CTIMP using Ravicti in cystic fibrosis patients will use 17 ml (18.7 g) or 25.5 ml (28.0 g) for 7 days (16). A CTIMP using 4PBA in α -1-antitrypsin patients dosed up to 20 g/day for 14 days (equivalent to 17.2 ml 18.9 g Ravicti) (10). A planned CTIMP using Ravicti in *ATF6*-related achromatopsia patients is proposing doses of 4.5 to 11.2 ml/m²/day (5 to 12.4 g/m²/day) up to 17.5 ml/day (19 g/day) for 6 months (17).



Ravicti is more acceptable to patients over 4PBA in the tablet form because it is an oral, odourless, tasteless liquid and it is not associated with unpleasant side effects such as taste and body odour. Maximum dosing requires 18 ml/day rather than 38 Buphenyl tablets/day.

2.1 Assessment and management of risk

Ravicti was approved by the FDA in 2013, and by EMA in November 2015 and has an excellent safety profile. Ravicti liquid will be used in preference to Buphenyl tablets as it is more acceptable to patients due to being odourless and tasteless. Ravicti is approved for use in patients from the age of 2 months, who may then be on it for life.

Our laboratory studies of mutant bestrophin 1 expressed in cells determined that the effective concentration of 4PBA was 2.5 mM. This is higher than the equivalent maximum daily dose for humans. Consequently, the trial will dose at the maximum daily dose. This approach is consistent with that of other clinical trials using Ravicti e.g. (15-17).

Participants will be dosed based upon body surface area at 11.2 ml/m²/day (12.4 g/m²/day) given in 3 equally divided dosages rounded up to the nearest 0.5 ml for 7 days. The total daily dosage will not exceed 18 ml (19.8 g). This complies with the prescribing recommendations for patients naïve to 4PBA (15) and it is rounded to the nearest 0.5ml to make the withdrawal easier for the subject/carer. Seven days of Ravicti dosing has been chosen as this will be sufficient time to allow for protein turnover and allow new bestrophin 1 to be translated in the presence of Ravicti.

As there are no existing medications for the treatment of bestrophinopathies the use of Ravicti will increase the risk of an undesirable effect.

This trial is categorised as: Type B = Somewhat higher than the risk of standard medical care.

Ravicti and Buphenyl have been in clinical use for decades and have an excellent safety profile. Thus, the chance of an adverse or serious adverse event is low. This trial does not plan for any mitigations for such events.

The following risks have been identified:

Risk: Difficulty or errors in administering the medication. Ravicti is an oral liquid medicine, that needs to be kept at room temperature and requires aspiration of a prespecified amount from the bottle prior to ingestion, with a quantity of medicine remaining in the vial. Some patients may not be comfortable administering the medication or have dexterity issues.

Mitigation: Patients will be trained to dispense the medication. There will be a phone call during week one with the research team and participants will be able to contact the research team via telephone



throughout the trial. Participants will be asked to return opened vials with left over unused medication for drug accountability. Patients who do not have the dexterity to self-administer and do not have a carer to assist them will be excluded from the study.

Risk: Patients may be unwilling or unable to participate due to location. If there are insufficient patients from Greater Manchester to power the study additional patients will be invited from Leeds.

Mitigation: The number of study visits has been kept to a minimum e.g. the EOG baseline and first day of Ravicti administration occur on the same day. The study will reimburse participants for travel and parking costs.

Risk: Ravicti has known minor side-effects. However, Ravicti may also have major influence on the ability to drive and the use of machines due to causing dizziness or headaches.

Mitigation: Patients will be advised not to drive to their screening appointment, and this will be stated in the PIS. Patients will also be warned not drive or use machines if they experience these side effects.

3 OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

Research objective: To compare the effect of glycerol phenylbutyrate on the EOG LP:DT ratio in patients with bestrophinopathy.

3.1 Primary objective

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.

3.2 Secondary objectives

If there is a change in the EOG LP:DT ratio after dosing with glycerol phenylbutyrate, does it return to baseline 21 days (-7/+14) after cessation of treatment?



3.3 Outcome measures/endpoints

This study has a crossover design. Baseline data will be collected at Visits 2 and 4. Primary outcome data will be collected at Visits 3 and 5. Further follow-up data will also be collected at Visit 6.

Patients will be randomised to either sequence A (glycerol phenylbutyrate then placebo) or sequence B (placebo then glycerol phenylbutyrate).

A seven-day administration period has been chosen as the researchers have shown that the half-life for wild type bestrophin 1 is 7.4 h; this is considerably less for mutant proteins associated with ARB and BVMD (ranging from 2.5 h to 3.9 h). Thus, during the first one or two days of the dosing period trial the existing bestrophin 1 will be fully turned over. New bestrophin 1 will be produced in the presence of glycerol phenylbutyrate (or placebo) for the remaining 5 to 6 days of the dosing period; this will be long enough to determine whether bestrophin 1 activity, as determined by the EOG LP:DT ratio, has been increased.

Baseline EOG LP:DT will be obtained for each period at Visits 2 and 4. Comparisons will be made between baseline and measurements taken 7 days after treatment with either glycerol phenylbutyrate or placebo on Days Visits 3 and 5 respectively (Period 1: Day 1 to Day 7; Period 2: a 7 day window starting any time from Day 22 to Day 42). Each subject will serve as their own control in the utilised crossover study design.

3.4 Primary endpoint/outcome

EOG LP:DT ratio measured on Visits 3 and 5

3.5 Secondary endpoints/outcomes

EOG LP:DT ratio measured on Visits 2 and 4 (baseline periods 1 and 2) and at Visit 6.



3.6 Table of endpoints/outcomes

Objectives	Outcome Measures	Timepoint(s) of evaluation of this outcome measure
To compare the effect of glycerol phenylbutyrate on the EOG LP:DT ratio in patients with bestrophinopathy	EOG LP:DT ratio	Visit 3 and 5(periods 1 and 2).

Secondary Objectives	Outcome Measures	Timepoint(s) of evaluation of this outcome measure
To determine whether the EOG LP:DT ratio returns to baseline 21 days (-7/+14) after glycerol phenylbutyrate dosing (if it changes after glycerol phenylbutyrate dosing)	EOG LP:DT ratio	Visits 2 and 4 (Baseline for periods 1 and 2) Visit 6 (14-35days after end of period 2)

4 TRIAL DESIGN

The trial design will be a double-blind cross-over design where half of the participants will be randomised to take placebo in the first period and glycerol phenylbutyrate in the second, and the other half will take glycerol phenylbutyrate first and placebo second.

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.

The EOG will be recorded from each enrolled participant at five defined timepoints: Visit 2 (before dosing with glycerol phenylbutyrate/placebo starts); Visit 3 (after 7 days dosing with glycerol phenylbutyrate/placebo); Visit 4 (14-35 days after glycerol phenylbutyrate/placebo dosing stops and the day the second treatment dosing starts with placebo/glycerol phenylbutyrate as appropriate); Visit 5 (the day after the last day of placebo/glycerol phenylbutyrate dosing); Visit 6 (14-35 days after second placebo/glycerol phenylbutyrate dosing ends).

5 TRIAL SETTING

The trial will take place at a single NHS Trust (MFT). Within MFT is Manchester Royal Eye Hospital and the Manchester Centre for Genomic Medicine where patients will be seen, and all trial procedures will be conducted.



The Ophthalmology Department in The Leeds Teaching Hospitals NHS Trust have a cohort of BVMD and ARB patients and have agreed to provide assistance with contacting the relevant affected individuals for possible recruitment to the study (if required, to provide sufficient participants to power the study). In other words, Leeds Teaching Hospital, if required, will be a Participant Identification Centre (PIC) site for MFT.

All patients participating in the trial will undergo trial procedures at MFT only.

6 PARTICIPANT ELIGIBILITY CRITERIA

6.1 Inclusion criteria

- Participants capable of giving informed consent
- Age 18 - 65 years old
- Best corrected visual acuity recorded as better than hand movements at recruitment
- Clinical and molecular* diagnosis of ARB or BVMD
- Able to speak and understand English

*a molecular diagnosis in the participant or a first degree relative

6.2 Exclusion criteria

- Participation in other CTIMP in the last 12 weeks
- Pregnant or breast feeding
- Liver morbidity
- Treatment for acute hyperammonaemia
- Urea cycle disorder
- Known hypersensitivity to phenylbutyrate and any excipient (I.e. glycerol)
- Reduced phenylbutyrate absorption due to pancreatic insufficiency or intestinal malabsorption
- Unable to speak and understand English
- Contraindicated concomitant medications:
 - Treatment with Probenecid (which may inhibit the renal excretion of metabolites of 4PBA including phenylacetylglutamine and phenylacetate)
 - Treatment with drugs that are substrates of CYP3A4. As 4PBA weakly induces CYP3A4 in humans it may decrease the systemic exposure to drugs that are



- substrates of CYP3A4 causing their loss of efficacy (e.g., hormonal contraception, alfentanil, quinidine, cyclosporine)
- Treatment with Midazolam (4PBA can decrease the systemic exposure of midazolam)

All individuals will be considered for inclusion in this study regardless of age, disability, gender reassignment, marriage and civil partnership, pregnancy and maternity, race, religion and belief, sex, and sexual orientation except where the study inclusion and exclusion criteria EXPLICITLY state otherwise.

7 TRIAL PROCEDURES

All procedures will be performed in an NHS clinical setting by appropriately qualified staff with valid GCP as listed on the trial delegation log.

7.1 Recruitment

7.1.1 Participant identification

Participants will be identified by the clinical care team at MFT. The database of The North West Genomic Laboratory Hub will be used to find existing patients with relevant genetic test results. Subsequently, the clinical care records will be inspected to assess potential eligibility for the trial. If needed, similar processes involving mining of the North West Genomic Laboratory Hub database will be followed to identify potential participants who are under the care of the Leeds Teaching Hospitals NHS Trust.

Patients appearing eligible will be approached by the research team, PI or Co-I with the participant information leaflet and consent forms (once the trial is open). The initial contact may be made by telephone, email or post, as deemed appropriate by the clinician. The PI or Co-I will discuss the trial with each patient, answering any questions and outlining procedures and potential risks and benefits. Each patient will be allowed as much time as needed for them to decide whether or not they wish to participate.



Confirmation of eligibility for screening, and for participation will be done by the CI/PI. Advertising will not be used.

7.1.2 Consent

Informed consent is required for all participants in this trial. Informed consent is a process initiated prior to an individual agreeing to participate in a trial and continues throughout the individual's participation. Consent from the patient will be obtained prior to participation in the trial, after a full explanation has been given of the treatment options, including the conventional and generally accepted methods of treatment.

The PI/Co-I will take written informed consent from the patient before any trial procedures are initiated, providing a fully signed copy to the patient for their records. Potential participants will discuss with an investigator about the nature and objectives of the trial, and the possible risks associated with their participation.

After the participant has entered the trial, the clinician must remain free to give alternative treatment to that specified in the protocol, at any stage, if he/she feels it to be in the best interest of the participant. However, the reason for doing so should be recorded and the participant will remain within the trial for the purpose of follow-up and data analysis according to the treatment option to which they have been allocated. Similarly, the participant remains free to withdraw at any time from the protocol treatment and trial follow-up without giving reasons and without prejudicing the further treatment.

7.1.3 Payment

There are no payments to patients for participation, however travel/subsistence costs will be covered.

7.2 Screening

Subjects who consented to take part in the study will be offered a screening appointment (Visit 1) during which there is the opportunity for further study discussion.

At this stage subjects will be allocated a screening number which will start at S01 and will progress in ascending numerical order such as S02, S03 etc. A screening number will be used until randomisation number will be allocated.

Screening-related procedures are outlined in the Trial Flow Chart (page 11) and will include: collection of clinical information (medical history, concomitant medication), pregnancy test, liver function test and



visual acuity assessment. Routine care information will not be used to screen patients for eligibility. Individuals who do not meet eligibility criteria at time of screening will not be offered rescreening.

The study investigators will keep a screening log of all potential participants about whom they are notified, and the eventual outcome. Reasons for non-recruitment will be documented (e.g. not eligible, declined consent etc.) and the information will be used for monitoring purposes. Patients do not need to provide a reason for non-consent. However, patients will be asked if they would like to provide a reason.

7.3 Randomisation

A participant may only be randomised once full eligibility has been confirmed by PI or a delegated member of the team and written informed consent has been obtained.

Patients will be randomised in a 1:1 ratio to either group 1 (glycerol phenylbutyrate then placebo) or group 2 (placebo then glycerol phenylbutyrate).

7.3.1 Method of implementing the randomisation/allocation sequence

Patient randomisation will be set up using www.sealedenvelope.com with a simple randomisation method and single block size by the Trials pharmacist. Screened subjects who pass eligibility criteria will be randomised in sequential order as per the devised method by the trial pharmacy. Once randomised, a three-digit randomisation number will be also assigned to participants and will run in numerical order starting from R01 and ending to R12.

The Trials pharmacy will hold a copy of the patient randomisation list and will be unblinded to the treatment allocation throughout the study. Both the participant and the members of the research team assessing the clinical outcomes will be blinded to the treatment arm.

At visit 2 full eligibility will be confirmed in writing by the PI or a delegated member of the team to clinical trial pharmacy. Once confirmation is received, an authorised member of the clinical trial pharmacy team will assign a treatment arm and a randomisation number to the participant as per patient randomisation list. The randomisation number will be also shared with the rest of the clinical and data analysis team via email.

7.4 Blinding

This will be a double-blind study. The placebo and active treatments will appear identical and will be dispensed in identical containers with identical labelling. All trial participants, care providers, outcome



assessors and data analysts will remain blinded throughout the study. Pharmacy staff will remain unblinded and will be responsible for both randomisation and maintaining the study blinding plan. For unblinding procedures see following section.

7.5 Unblinding

Unblinding of participants during the conduct of the clinical trial will not be allowed unless the treating physician believes there is a compelling reason to do so. Unblinding may be used to enable treatment of serious adverse event/s, or to enable administration of another therapy that is contraindicated by the trial treatment. The decision to do so would sit with the treating physician, PI, and/or investigators.

In the event of an emergency, the decision to unblind a participant resides with the CI/PI and all investigators on the study delegation log. CI/PI/clinical cover can be reached 24 hours a day via MFT switchboard by either a clinical staff member (i.e. doctor, pharmacist) or directly by the participants. Contact details of the MFT switchboard are included on the patient's diary which will be handed to the subject at time of enrolment and can also be accessed online.

During working hours, if the investigators on the study make the decision to unblind a participant, they can contact the clinical trials pharmacy team who store the unblinding list and can reveal the treatment allocation. The list is held separately from the rest of the study documentation to avoid accidental unblinding.

If emergency unblinding is required during out-of-hours, those investigators delegated can access the unblinding list via the on-call pharmacist. On call pharmacy cover is provided 24 hours a day and 7 days a week. Information on how to access the trial blinding information will be held by the pharmacy department and all on-call pharmacists will know how to access it. The MFT Pharmacy is familiar with such procedures from many previous clinical trials conducted at MFT.

Non-emergency unblinding

For non-emergency unblinding requests, the ward pharmacist or delegate will contact the trial pharmacy team. The trial pharmacy team will then liaise with the CI to confirm whether unblinding is necessary. If the CI confirms that unblinding is deemed necessary, the treatment allocation is revealed to the individual who issued the unblinding request by clinical trial pharmacy.

7.6 Baseline data



- At Visit 1, following consent and enrolment, the following data will be collected. All EOG and ERG measurements will be taken at the same time each day (morning or afternoon), by a member of the clinical electrodiagnostic staff:
- Medical history
- Weight and height (for body surface area and dosage calculations)
- Concomitant medications check
- Liver function test
- Pregnancy test
- Visual acuity (VA)

The following baseline data will be collected on Visit 2 which is max 7 days after Visit 1:

- ERG
- EOG
- FAF
- OCT

7.7 Trial assessments

All tests and assessments will be conducted using national standards and the Standard Operating Procedures (SOP) of the Manchester Royal Eye Hospital (MREH). All EOG and ERG measurements will be taken at the same time each day (morning or afternoon), by a member of the clinical electrodiagnostic staff.

- Pregnancy test (visits 1 and 6)
- Liver function test (visits 1, 3, 4, 5 and 6)
- Drug diary check (visits 3, 4, 5 and 6)
- Visual acuity (visits 1 and 6)
- Electrophysiology (ERG and EOG; visits 2, 3, 4, 5 and 6)
- Ophthalmic imaging (FAF and OCT; visits 2, 3, 4,5 and 6)

The EOG test at the end of dosing must be immediate (as scheduled) to avoid missing any effect the drug treatment has on the EOG. Any delay means new bestrophin 1 protein will be made in the absence of the drug and this may affect the EOG.



To mitigate the occurrence of protocol deviations, dates will be confirmed with the participant immediately prior to dosing so there is high confidence that they can attend again at the end of dosing.



Schedule of events

Assessment	Visit 1 Consent and enrolment	Visit 2 Day 1	Visit 3 Day 8	Visit 4 14-35 days after Visit 3**	Visit 5 7 days after Visit 4	Visit 6 14-35 days after Visit 5**
Molecular confirmation of BVMD or ARB diagnosis (from existing data)	x					
Informed consent and enrolment	x					
Height, weight, surface area & dosage calculations	x					
Liver function test	x		x	x	x	x
Pregnancy test	x					x
Medical history	x					
Concomitant medications check	x	x	x	x	x	x
VA	x					x
Randomisation		x				
SAE check		x	x	x	x	x
ERG*		x	x	x	x	x
EOG*		x	x	x	x	x
FAF		x	x	x	x	x
OCT		x	x	x	x	x
Dosing (7 days)		x		x		

* EOG and ERG measurements will be taken at the same time each day (morning or afternoon), by a member of the clinical electrodiagnostic staff.

** Visits 4 and 6 follow a washout period. Ideally this will be 21 days after the previous visit but can be between 14 and 35 days after the previous visit if necessary to accommodate the availability of a participant. This will have no adverse effect on the conduct of the trial.

7.8 Withdrawal criteria



A participant will be withdrawn from the study if any of the following applies:

- They choose to withdraw
- Following safety review by the TSC
- Severe disease where palliative care is adopted
- In case of intentional overdose
- Abnormal liver function test
- Deleterious change in vision
- Pregnancy occurs during treatment
- Unacceptable side effects

A participant will not remain in the study if the IMP is discontinued.

7.9 End of trial

The trial will end after the last participant's final visit which is scheduled to be within 18 months of the start date of the trial. The end of study report will be submitted within 12 months of the end of the trial.

7.10 Samples

Participants will have a liver function test at visits 1, 3, 4, 5 and 6, and a pregnancy test at visits 1 and 6. These will be done as part of standard care, by clinical staff and sent to standard Trust labs for analysis. Results will be added to the participant's electronic record as usual, and the research team will review results and manually transcribe the data into the study CRF. The samples will be destroyed as per standard Trust SOPs.

8 TRIAL TREATMENTS

The trial is a double-blinded study with a cross-over design. Half of the subjects will be randomised to take placebo in the first period and glycerol phenylbutyrate in the second. The remaining half will be randomised to glycerol phenylbutyrate in the first period and placebo in the second. Therefore, all participants will have received a treatment course of active substance and placebo at the study completion. For the purpose of this trial, the IMP refers to either glycerol phenylbutyrate or placebo. A full description of the active substance and placebo is provided in the following subsection.



8.1 Name and description of investigational medicinal products

Active substance: Glycerol phenylbutyrate oral solution supplied as 1.1 g of glycerol phenylbutyrate per ml (25ml bottles). Glycerol phenylbutyrate is licenced in the UK under the brand name of Ravicti and it is manufactured by Immedica. The clinical formulation of glycerol phenylbutyrate is detailed in the SmPC.

Placebo: Glycerol oral solution supplied as 100% v/v (25ml bottles).

IMP Manufacturing: Both active substance and placebo used in this study will be manufactured by IPS Pharma.

Glycerol phenylbutyrate will be purchased from Immedica and relabelled by IPS pharma according to the blinding requirements set for this study. The marketed label will be removed and the trial label will be applied on the Ravicti bottle. The bottle will not be opened; therefore, final dosage, pharmaceutical form, chemical properties and excipient content will not be altered.

Glycerol will be sourced via AAH wholesaler and bottled by IPS Pharma in the same container used for glycerol phenylbutyrate. Bottles will be purchased from the same distributor used by Immedica for Ravicti.

For blinding purposes, both glycerol phenylbutyrate and placebo will be packaged and labelled in an identical manner by IPS Pharma. Further details of the manufacturing process, including a summary of product characteristics, packaging and labelling of both IMPs provided in this study will be described in the simplified IMP Dossier (sIMPD).

8.2 Regulatory status of the drugs

Marketing authorisation of glycerol phenylbutyrate: Immedica Pharma AB. SE-113 63 Stockholm. Sweden. Authorisation number: EU/1/15/1062/001

Glycerol phenylbutyrate is currently licensed in the UK with a restricted specialist use for the treatment of hyperammonaemia due to UCD. In this study, glycerol phenylbutyrate will be re-purposed for the treatment of bestrophenopathy due to its secondary action in restoring cellular function of mutant proteins. Glycerol phenylbutyrate will be supplied in its marketed container (glass bottle); however, both brand label and outer carton will be removed to adhere with the trial requirements of this study.

Marketing authorisation of Glycerol: L.C.M. Ltd. Linthwaite Laboratories. Huddersfield HD7 5QH England. Authorisation number: 12965/0012



Glycerol oral solution 100% v/v is currently licensed in the UK and used in clinical practice for the treatment of sore throats.

IPS pharma will be responsible for all the manufacturing steps including re-labelling.

8.3 Product Characteristics

The trial will use the SmPC as Reference Safety Information. SmPC updates will be identified by the CI and the sponsor annually.

8.4 Drug storage and supply

8.4.1 Drug storage

IMPs will be stored in the pharmacy department at the hospital site. Storage and shelf-life requirements for both IMPs are detailed in the sIMPD. Unopened bottles should be stored at room temperature in line with the SmPC. After the first opening of the bottle, the medicinal product must be used within 14 days.

8.4.2 Supply and packaging

The IMPs (glycerol phenylbutyrate and glycerol) will be supplied by IPS Pharma to the pharmacy departments at the hospital site. Both IMPs will be delivered in a single shipment to the hospital site before the first subject is enrolled into the study. As a result, ordering of extra supply of IMPs is not required. The IMPs will be stored in the clinical trial pharmacy and temperature monitored 24 hours a day. Pharmacy will acknowledge the receipt of the IMPs arrival at the hospital site on the master accountability log and is responsible for keeping this document up to date throughout the study by updating the balance of the IMPs at each dispensing visit.

The IMPs will be packaged in a clear, Type III glass, bottle with a high-density polyethylene (HDPE) child-resistant closure with integrated syringe insert. Each bottle will contain 25 ml of liquid. Each bottle will be labelled and packaged according to Good Manufacturing practice (GMP) and Good Clinical practice (GCP) and Annex 13. Each final product batch will be supplied to the hospital site with the Qualified Person (QP) release certificate documentation.



8.4.3 Accountability and destruction

All movements of study medication (glycerol phenylbutyrate or glycerol) between IPS Pharma and hospital site will be documented on both master and patient specific accountability logs. The investigator will prescribe the number of bottles required for each participant at the study visit. Participants will be asked to bring all unused medication to the clinical team at the follow-up visits. All unused medication will be returned to the pharmacy department at the hospital site who will acknowledge returns on the accountability logs and report the number of bottles returned by each participant. Destruction will take place after reconciliation of batches and will be the responsibility of the pharmacy department at the hospital site under the guidance of the Sponsor. The hospital site will only do this after receiving written approval from the Sponsor. Proof of disposal will be provided by the site to the Sponsor.

8.4.4 Arrangements for post-trial access to IMPs

At the completion of the study, participants will stop having access to the study medications (glycerol phenylbutyrate and glycerol) for free. It is the responsibility of the PI to explain post-trial arrangements with the subjects. The duration of treatment will be indicated in the patient consent form. At the final visit the study doctor will discuss the choices for the future medical care with each subject.

8.5 Preparation and labelling of Investigational Medicinal Products

IMPs (glycerol phenylbutyrate and glycerol) will be supplied to the clinical site with the Annex 13 complaint label. IMPs (glycerol phenylbutyrate or glycerol) will be dispensed at visits 2 and 4.

Participants or a carer will be trained on how to prepare and administer the dispensed IMP. Participants will self-administer the prescription at home. Oral syringes will be supplied by the pharmacy at each dispensing visit.

8.6 Dosage schedules

Participants will be dosed based on body surface area at a dose of 11.2 ml/m²/day (12.4 g/m²/day). Any formula can be used to calculate body surface area. Dose will be calculated at visit 2 and will remain the same throughout the study. Two doses will be taken on the first dosing day and



subsequently in three equally divided dosages per day rounded up to the nearest 0.5 ml. The final dose will be taken on the morning of the assessment (to ensure there is not a large interval between dosing and assessment). The total daily dosage will not exceed 18 ml (19.8 g). Each dose will be taken orally and may be added to a small amount of apple sauce, ketchup, or squash puree. Oral syringes should not be rinsed with water between daily doses and a new syringe should be used each day as presence of water can cause degradation of glycerol phenylbutyrate. Each subject will administer study medication themselves and will be given sufficient IMP to take for 7 days at home. The participants will be asked to keep a dosing diary recording the date and time of medication taken. The dosing diary and any remaining medication will be checked at the final study visit (Visit 6).

If a dose is missed, the missed dose should be taken as soon as the participant remembers. However, if the next dose is due in less than 2 hours, then the missed dose should be skipped and the next dose taken as normal.

8.7 Dosage modifications

There will be no dosage modifications other than complete withdrawal from the trial.

8.8 Known drug reactions and interaction with other therapies

Undesirable effects

The following observations are based on exposure of 65 adult UCD patients across four short term and three long term clinical studies. At the beginning of the treatment, abdominal pain, nausea, diarrhoea, and/or headache may occur; these reactions usually disappear within a few days even if treatment is continued. The most frequently reported adverse reactions (>5%) during glycerol phenylbutyrate treatment were diarrhoea, flatulence, and headache (8.8% each); decreased appetite (7.0%); vomiting (6.1%); fatigue, nausea and, abnormal skin odour (5.3% each).

Abnormal skin/body odour, probably caused by the metabolite phenylacetate, is commonly reported. Amenorrhoea or irregular menstruation is very common. Reported haematological disturbances include anaemia, thrombocytopenia, leukopenia, thrombocytosis, and leucocytosis. Electrolyte disturbances, metabolic acidosis, alkalosis, and renal tubular acidosis are common. Increases in hepatic enzymes, bilirubin, and uric acid can occur.



Special warnings and precautions for use

Neurotoxicity

Reversible clinical manifestations suggestive of neurotoxicity (e.g., nausea, vomiting, somnolence) have been reportedly associated with phenylacetate levels ranging from 499-1285 µg/ml in cancer patients who received phenylacetic acid (PAA) intravenously. Although these have not been seen in clinical trials involving UCD patients, high PAA levels should be suspected in affected individuals (particularly in children < 2months) with unexplained somnolence, confusion, nausea and lethargy who have normal or low ammonia.

If symptoms of vomiting, nausea, headache, somnolence, confusion, or sleepiness are present in the absence of high ammonia or other intercurrent illnesses, then the participant will be withdrawn from the study.

Other warning and precautions

The efficacy of glycerol phenylbutyrate may be reduced in patients with pancreatic insufficiency or with conditions of intestinal malabsorption.

Driving and machine use

Glycerol phenylbutyrate may have major influence on the ability to drive and use machines given that treatment with glycerol phenylbutyrate may cause dizziness or headaches. Patients should not drive or use machines if they experience these side effects.

8.9 Concomitant medication

Potential for other medicinal products to affect ammonia:

- Corticosteroids: Use of corticosteroids may cause the breakdown of body protein and increase plasma ammonia levels. Ammonia levels need to be monitored closely when corticosteroids and glycerol phenylbutyrate are used concomitantly.
- Probenecid: Probenecid may inhibit the renal excretion of metabolites of glycerol phenylbutyrate including phenylacetylglutamine.



Concomitant use of medicinal products known to inhibit lipase should be given with caution as glycerol phenylbutyrate is hydrolysed by digestive lipase into phenylbutyrate acid and glycerol. This may be associated with increased risk of medicinal product interactions with lipase inhibitors and with lipase contained in pancreatic enzyme replacement therapies.

A potential effect on CYP2D6 isoenzyme cannot be excluded and caution is advised for patients who receive medicinal products that are CYP2D6 substrates.

Glycerol phenylbutyrate is known to be a mild activator of CYP3A4 and thus it may reduce efficacy of medicinal products which are a substrate of the CYP3A4 (including hormonal contraception). Concomitant administration should be avoided and has been included as an exclusion criteria for this study.

8.10 Trial restrictions

Effective contraceptive measures must be taken by women of child-bearing potential. Women of child-bearing potential is defined by 'fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy.

Glycerol phenylbutyrate should not be used during pregnancy and in women of childbearing potential not using contraception, unless the clinical condition of the woman requires treatment with glycerol phenylbutyrate. It is unknown whether glycerol phenylbutyrate or its metabolites are excreted in human milk. A risk to the new-borns/infants cannot be excluded. Therefore, glycerol phenylbutyrate will not be used during breastfeeding.

Female participants of childbearing capacity will have a serum pregnancy test performed at Visit 1 (pre-dosing) and at Visit 6 (end of study and 3 weeks after the last IMP administration).

In addition, they must agree to maintain highly effective contraceptive measure during the study. The European Union Clinical Trial Facilitation Group guidance document has defined 'highly effective contraception' methods as follows:

- Combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, or transdermal)
- Progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, or Implantable)
- Intrauterine device



- Intrauterine hormone-releasing system
- Bilateral tubal occlusion
- Vasectomised partner
- True sexual abstinence (when this is in line with the preferred and usual lifestyle of the participant).

Periodic abstinence is not an acceptable form of highly effective contraception.

Due to the possible interaction of glycerol phenylbutyrate with CYP3A4, the addition of a barrier method is also mandatory in case hormonal contraception is preferred (i.e. existent contraception) as the therapeutic effects of some oral contraceptives that are substrates of this enzyme may be reduced. The barrier method should last whilst taking the study medicine and for at least 28 days after stopping it.

8.11 Assessment of compliance with treatment

Participants will be asked to keep a dosing diary recording the date and time of medication taken from visits 2 to visit 3 and from visit 4 to visit 5. All unused medication will be returned to the pharmacy department at the hospital site at the follow-up visits after the end of a dosing period on visit 3 and visit 5, at which times the dosing diary will be checked.



9 PHARMACOVIGILANCE

9.1 Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	<p>An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant.</p> <p>The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out.</p> <p>All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions. It is important to note that this is entirely separate to the known side effects listed in the SmPC. It is specifically a temporal relationship between taking the drug, the half-life, and the time of the event or any valid alternative etiology that would explain the event.</p>
Serious Adverse Event (SAE)	<p>A serious adverse event is any untoward medical occurrence that:</p> <ul style="list-style-type: none"> • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect <p>Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences.</p> <p>NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.</p>
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.



Suspected Unexpected Serious Adverse Reaction (SUSAR)	<p>A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out in the reference safety information:</p> <ul style="list-style-type: none"> • in the case of a product with a marketing authorisation, this could be in the summary of product characteristics (SmPC) for that product, so long as it is being used within its licence. If it is being used off label an assessment of the SmPCs suitability will need to be undertaken. • in the case of any other investigational medicinal product, in the investigator’s brochure (IB) relating to the trial in question
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9.2 Operational definitions for (S)AEs

As this is a CTIMP with a licensed IMP used outside of its licensed indication, all SAEs, SARs and SUSARs will be reported to the sponsor. AEs and ARs will be reported in the CRF via the MFT AE form.

AEs will be described as ‘mild’ if there is no need for intervention, ‘moderate’ if medication is required for relief and ‘severe’ if discontinuation or temporary interruption of glycerol phenylbutyrate is needed. The latest MHRA-approved SmPC will be used as Reference Safety Information to determine the anticipated nature of ARs and SARs.

9.3 Recording and reporting of SAEs, SARs AND SUSARs

All SAEs will be reported, via the trial-specific SAE form, by email (Adverse.Events@mft.nhs.uk) to the study sponsor within 24 hours of the PI or site research team becoming aware of them. The site PI should not wait until all information concerning the event is available, as this may be documented later on a follow-up SAE form. Follow-up information should be documented and communicated to the study sponsor as soon as it becomes available. Follow-up information should include whether the SAE has resolved/persists, if and how it was treated and whether a patient continues in the study or not. The PI will ensure all SAEs are followed up until their resolution or the follow-up is complete.

The CI and sponsor will ensure SAEs are reported annually to the appropriate ethics committee, the MHRA and the TSC. SAEs suspected to have occurred from administration of a study intervention but that are also “unexpected” in terms of the existing information and clinical experience of the medicinal product in question are known as “suspected unexpected serious adverse reactions” (SUSARs). SUSARs which are either fatal or life-threatening will be reported to the MHRA within 7 days of the sponsor being aware of their occurrence. Any SUSARs that are not fatal, nor considered life-threatening will be reported to the MHRA within 15 days of the sponsor becoming aware of them. If the death of a participant is deemed potentially related to participation in this trial, then it will be reported



immediately to the study sponsor and subsequently to the MHRA according to the timeframes described above.

9.4 Responsibilities

This section summarises the responsibilities for reporting and reviewing toxicity and safety information arising from the trial and any timeline associated with these.

Principal Investigator (PI)

The PI (and, in the absence of the PI, a co-investigator who will also be a medic) will be responsible for detection of AEs and ARs suffered by participants throughout the intervention and follow-up periods, as well as:

1. Using medical judgement in assigning seriousness, causality and whether the event/reaction was anticipated using the Reference Safety Information in the IMP's summary of product characteristics.
2. Ensuring that all SAEs are recorded and reported to the sponsor within 24 hours of becoming aware of the event and providing further follow-up information as soon as available. Ensuring that SAEs are chased with Sponsor if a record of receipt is not received within 2 working days of initial reporting.
3. Ensuring that AEs and ARs are reported in the CRF via the MFT AE form.



Chief Investigator (CI; as this is single centre study the CI and PI will be the same person)

The CI will be responsible for the clinical oversight of the safety of trial participants, including an ongoing review of the risk / benefit.

1. Immediate review of all SUSARs.
2. Review and reporting of all safety information to the Trial Steering Committee
3. Assigning Medical Dictionary for Regulatory Activities (MedDRA) or Body System coding to all SAEs and SARs.
4. Preparing the clinical sections and final sign off of the Development Safety Update Report (DSUR).

Sponsor

The sponsor will take responsibility for:

1. Central data collection and verification of SAEs, SARs and SUSARs according to the trial protocol onto a database.
2. Expedited reporting of SUSARs to the Competent Authority (MHRA in UK) and REC within required timelines.
3. Checking for (annually) and notifying PIs of updates to the Reference Safety Information for the trial.
4. Preparing standard tables and other relevant information for the DSUR in collaboration with the CI and ensuring timely submission to the MHRA and REC.

Trial Steering Committee (TSC)

In accordance with the written Terms of Reference, the TSC will periodically review cumulative safety data and liaise with the sponsor and MHRA regarding safety issues. The TSC charter will include details of the committee's membership, remit and schedule of meetings.

9.5 Notification of deaths

Any deaths, whether assessed to be caused by the IMP or not, will be reported to the sponsor. This report will be immediate.



9.6 Pregnancy reporting

- All pregnancies within the trial (either the trial participant or the participant's partner, with participant's consent) should be reported to the CI and the Sponsor using the relevant Pregnancy Reporting Form within 24 hours of notification.
- Pregnancy is not considered an AE unless a negative or consequential outcome is recorded for the mother or child/foetus. If the outcome meets the serious criteria, this would be considered an SAE.
- Follow-up of pregnant participant: a pregnant participant will be followed until birth of the foetus and birth outcome recorded. Pregnancy information will be collected as required for reporting from standard of care medical records.

9.7 Overdose

An overdose is a deliberate or inadvertent administration of a treatment at a dose higher than specified in the protocol. All accidental or intentional overdoses will be recorded; this will usually be reported by the participant in their medication diary. If an overdose results in signs or symptoms that meet criteria for adverse event they will be reported as described in section 9.3. A participant will be withdrawn from the study in the event of intentional overdose.

9.8 Reporting urgent safety measures

The sponsor and investigators may take appropriate urgent safety measures to protect clinical trial subjects from any immediate hazard to their health and safety. If any urgent safety measures are taken the CI/Sponsor shall immediately, and in any event no later than 3 days from the date the measures are taken, give written notice to the MHRA and the relevant REC of the measures taken and the circumstances giving rise to those measures.



9.9 Development safety update reports

The CI will provide (in addition to the expedited reporting above) DSURs once a year, in collaboration with the sponsor, throughout the clinical trial, or as necessary, to the MHRA, the sponsor and where relevant the REC. The report will be submitted within 60 days of the anniversary of the CTA for the trial, for every year the trial is active until the trial is declared ended.

10 STATISTICS AND DATA ANALYSIS

A full Statistical Analysis Plan will be produced for the trial, with a summary below.

10.1 Sample size calculation

The primary outcome is LP:DT ratio at visit 3 or visit 5. 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).

10.2 Planned recruitment rate

This study will recruit from MFT, with additional patients referred from Leeds if required. It is estimated this trial will fully recruit to the target of 12 participants within a period of 6 months.

10.3 Statistical analysis plan

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.

10.3.1 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 at visits 2 and 4. These will be descriptively compared using summary statistics and boxplots. All baseline data will be reported overall and for the two randomisation arms.

10.3.2 Primary outcome analysis

For the primary analysis, outcomes at visit 3(period 1) and 5 (period 2) will be reported descriptively by treatment. Baseline EOG will be obtained for each period at visits 2 and 4 respectively. Comparisons will be made between baselines for period 1 and visit 3, and for period 2 and visit 5, 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.

10.3.3 Secondary outcome analysis

Secondary analyses will explore the trajectory over time from baseline to Visit 6. 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 visit 2 and 4 and the visit 6 EOG using descriptive statistics and graphics.

10.4 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.

10.5 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.

10.6 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. This criteria may include safety issues, noncompliance, significant concerns (e.g., complaints by a participant or any other party), or an inspection finding(s).

10.7 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.

10.8 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 visit 2 or 4.

11 DATA MANAGEMENT



11.1 Data collection tools and source document identification

Data will be collected and handled with prior written consent, in compliance with GCP (Good clinical Practice), General Data Protection Regulation and the Data Protection Act (2018), and MFT SOPs regarding data management. Specifically, the data collected for the study will have the following source data from patients NHS medical records:

- Demographic data including name, date of birth, medical history, genetic test reports, ocular imaging data.

The following new source data will be generated during the trial:

- Ocular imaging and EOG data, consent paperwork, weight measurement, blood test results, medication dosing records.

All data will be pseudonymised using a unique ID which only the study team members involved with patient care will have access to (and so be able to link data to the patient). All paper records will be stored in locked filing cabinets within swipe-card access areas of the Trust. All electronic records will be stored in password protected folders hosted on the Trust server, protected by the N3 firewall. A fully anonymised copy of the data will be stored on the study statistician's personal area of University of Manchester servers for analysis purposes only.

No identifiable data will pass outside of the study team as listed on the delegation log. There will be no transfer of data outside the UK. Any published findings will use the unique participant ID only.

Source Data

Please see corresponding data management plan and source data location log. Source data will be taken from:

MFT HIVE electronic health care record

MREH imaging resources

All data will be collated on a specifically designed and validated Excel database. The study Project Manager will be responsible for data entry. Data checks for quality and consistency will be performed as per the full Data Management Plan. All data will be pseudonymised.



11.2 Access to Data

Paper records will be kept in locked drawers/filing cabinets in swipe card access hospital departments.

Electronic records will be password protected and only accessible to MFT staff and affiliates listed on the delegation log.

11.3 Archiving

Archiving of paper material (ISF etc.) will be for a term of 25 years in line with MFT SOPs.

MFT archiving is serviced by RESTORE.

12 MONITORING, AUDIT & INSPECTION

The study will be subject to the audit and monitoring regime of MFT in line with applicable MFT SOPs and policies. The study will be subject to a monitoring plan maintained by the MFT Quality Team and will also have an annual survey sent out for completion by a member of the research team.

The CI will ensure access to source data and documents for trial related monitoring by a representative of the study sponsor (MFT).

13 ETHICAL AND REGULATORY CONSIDERATIONS

13.1 Regulatory review and compliance

Before the start of the study, a favourable opinion will be sought from an NHS Research Ethics Committee (REC), the HRA and letter of acceptance (clinical trial authorisation) from the MHRA for the study and all the supporting documents including the protocol, information sheets, informed consent forms and other relevant documents. The study team will be responsible for the maintenance of a study site file, the sponsor will maintain the TMF, in which all current and superseded study documents will be retained. Also contained in the site file/TMF will be the approval documentation including correspondence with relevant authorities such as the MHRA, HRA and REC.

The study team are responsible for producing progress reports throughout the study, including annual reporting (APR) to REC as required. The Chief Investigator will notify the REC and the MHRA of the end of the study, and will submit a final report with the results, including any publications/abstracts, to



the REC & MHRA within 12 months of the end of the study. If the study is ended prematurely, the Chief Investigator will notify the REC and MHRA including the reasons for the premature termination.

No participants will be enrolled into this research study prior to the study being reviewed by the relevant regulatory authorities and receiving MHRA, HRA and REC approvals, as well as approval from the R&D office at Manchester University NHS Foundation Trust and green light from the study Sponsor.

13.2 Peer review

The grant proposal for the concept of this study has been peer reviewed as per MRC application process.

13.3 Public and Patient Involvement (PPI)

Best efforts will be made to involve patients with bestrophinopathy to ensure research relevance. However, given the rarity of the studied condition a more general patient population may be required. Patients will lead on producing the trial materials including participant information sheet (PIS) and informed consent form, using appropriate templates. The Manchester Royal Eye Hospital (MREH) and Manchester Centre for Genomic Medicine have contacts for previous PPI participants. With the MREH Clinical Trials Manager the researchers will set up a PPI group to be managed by patients/trial staff for participants to feedback their experience of trial participation. Participants will receive recompense in accordance with National Institute for Health and Care Research 'Briefing notes for researchers - public involvement in NHS, health and social care research' (<https://www.nihr.ac.uk/documents/briefing-notes-for-researchers-public-involvement-in-nhs-health-and-social-care-research/27371>) and local guidelines.

13.4 Amendments

Any amendments to the study shall be reviewed by the Sponsorship Team prior to submission. Any non-substantial amendments shall be notified to the HRA and any substantial amendments, along with amended documentation, shall be approved by the MHRA, REC, and HRA, prior to implementation as per nationally agreed guidelines. The Chief Investigator or designee will work with the R&I department to put the necessary arrangements in place to implement the amendment and to confirm their support for the study as amended.



13.5 Protocol compliance

- The research team will be vigilant in protocol deviations and will record them on a study specific deviation log which will be regularly assessed by the PI.
- Deviations that may affect the safety, physical or mental integrity of participants or scientific value of the study will be reported to the study sponsor via research.sponsor@mft.nhs.uk by the research team.
- Deviations from the protocol which are found to frequently recur are not acceptable, will require immediate action and could potentially be classified as a serious breach and should also be reported to the sponsor without delay.

13.6 Notification of Serious Breaches to GCP and/or the protocol

A “serious breach” is a breach which is likely to effect to a significant degree –

- (a) the safety or physical or mental integrity of the participants of the trial; or
 - (b) the scientific value of the trial
- the sponsor will be notified immediately of any case where the above definition applies during the trial conduct phase
 - the sponsor of a clinical trial will notify the licensing authority in writing of any serious breach of
 - (a) the conditions and principles of GCP in connection with that trial; or
 - (b) the protocol relating to that trial, as amended from time to time, within 7 days of becoming aware of that breach.

13.7 Data protection and patient confidentiality

All investigators and trial site staff must comply with the requirements of the Data Protection Act 2018 with regards to the collection, storage, processing and disclosure of personal information and will uphold the Act’s core principles.



The collection, storage (place and duration), maintenance, access, and security of the data associated with this trial, and data details of the data custodian, are described in the trial's data management plan.

13.8 Financial and other competing interests for the chief investigator, PIs at each site and committee members for the overall trial management.

The manufacturer, supplier and distributor of the IMP used for this study have had no influence on the design or drafting of this study protocol, neither will they play any part in the direct conduct of the study, analysis or publication of results. None of the investigators listed in this protocol have any competing interests to declare.

13.9 Indemnity

The NHS indemnity scheme will apply to this study to ensure it meets the potential legal liability of the sponsor, equipment, employer and investigators/collaborators for harm to participants arising from the management, design and conduct of the research. No arrangements will be made for the payment of compensation in the unlikely event of harm.

13.10 Post trial care

Patients will return to NHS standard of care.

13.11 Access to the final trial dataset

The CI and the sponsor will have access to the final dataset. The final dataset will be stored pseudoanonymised using study codes, as a single data spreadsheet at MFT.

The pseudoanonymisation key will be stored on MFT servers behind the Trust's firewall, in a separate location from the data spreadsheet, in a password protected PC. The study statistician will not have access to the patient identification record. They will receive an anonymised version of the data for analysis which will be stored on their personal area on University of Manchester servers. The study ID will be removed and replaced with a unique sequential number beginning with 1.



14 DISSEMINATION POLICY

14.1 Dissemination policy

On completion of the study, the data will be analysed and tabulated, and a final study report prepared. The study will be registered with the ISRCTN registry where the trial report can be accessed.

The results of this study will be disseminated to local stakeholders and to the wider research community. Local stakeholders include the patients participating in the trial and relevant clinicians in MREH. The former will be informed of the outcome via a post-trial meeting at MREH during which there will be a presentation by the trial clinicians and scientists. A summary patient information handout using lay language will be produced. If the outcome of the trial is positive this handout may be distributed to a wider audience e.g. trial participants in a future trial.

Any publishable scientific findings of interest to the field will be published in the scientific literature with open access following peer review.

14.2 Authorship eligibility guidelines and any intended use of professional writers

The final trial report will be co-authored by the trial clinicians and scientists who will each cover their own area of speciality. These include:

1. Dr Eva Lenassi (medical and ophthalmology)
2. Dr Forbes Manson (genetics and science of bestrophinopathies)
3. Prof Neil Parry (clinical electrophysiology (EOG and ERG))
4. Dr Catherine Fullwood (statistical analysis)
5. Dr Beatriz Duran Jimenez (pharmacy)
6. Dr Miriam Lettieri (pharmacy)



15 INTELLECTUAL PROPERTY

15.1 Foreground IP

This project is funded by the Medical Research Council and this grant is managed by University of Manchester, and under the MRC funding terms:

1. All intellectual property shall belong to the party that generates them.
2. Where the grant is associated with more than one Research Organisation and/or other project partners, the basis of collaboration between the organisations including ownership of intellectual property and rights to exploitation, must be set out in a formal collaboration agreement in a way that is proportionate to and appropriately reflects the exact nature of the collaboration.

This collaboration agreement was signed in December 2022 and confirms that MFT and UoM will each own any IP created by their own employees, unless it is IP that can't be separated, in which case it will be jointly owned.

15.2 Background IP

All Background IP used in connection with the Project shall remain the property of the Party contributing the same. The Parties agree that any Improvements will be deemed to form part of that Party's Background IP and be owned by that Party.

16 REFERENCES

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17. APPENDICIES

17.1 – Schedule of Procedures

17.2 Amendment History

Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
1	1.1	15/05/2024	Sarah Rushton	Update to the participant drug diary. The emergency telephone details have been updated to include further detail on contacting the study team both inside and outside of office hours. New version: BETTER Drug diary_v1.1 15.05.2024
2	1.2	13/02/2024	Sarah Rushton	<p>1 - A window has been added to visits 4 and 6 to add incase flexibility is required to accommodate the availability of the participant.</p> <p>2Clarification that the clinician/research team may initially approach the patient by telephone or email at the time of sending the study information, as deemed appropriate by the clinical team.</p> <p>3 - Study end date adjusted to 30.04.2025 due to the study opening later than planned. No change to the study duration.</p> <p>4 - The protocol has been adjusted to version 1.2 (31.05.2024) in line with the above, plus small administrative changes.</p> <p>5 - Leeds Teaching Hospitals NHS Trust added as a PIC site.</p> <p>6 - New Study invitation templates to accompany the participant information sheet - BETTER invitation letter v1.0 (31.05.2024) and BETTER invitation email v1.0 (31.05.2024)</p>
	1.3	26/07/2024	Neda Shahedy, Karen Tricker	Rationalised text in protocol, relating to increased flexibility to washout period to ensure all references to this are consistent.



				<p>Formatting and page numbers corrected in protocol.</p> <p>New BETTER PIS updated in line with changes from the protocol amendment 1.2</p>
3	1.4	29/10/2024	Neda Shahedy, Karen Tricker, Sarah Rushton	<p>1 - Protocol updated to v1.4 29.10.2024. Amendment to protocol includes removing the requirement for first dose to be taken on site (as this is not clinically necessary) and to clarify that the first two doses are taken on the first dosing day, and the final dose is taken on the morning of the assessment. This is to ensure that the participant can allow at least 3 hours between doses, and there is not a large interval between the final dose and assessment. The protocol has also been updated to reflect the other changes listed in this amendment.</p> <p>2 - Drug diary has been amended to reflect the changes outlined in the protocol (as shown in change 1). Drug diary updated to v1.2 29.10.2024.</p>
4	1.5	14/03/2025	Sarah Rushton, Neda Shahedy	<p>1 – Study extended to end of December 2025 due to confirmation of extension from the MRC and budget supplement (£5,691.20).</p> <p>2 – Protocol updated to v1.5 (14.03.25) in line with extension to end date.</p>
5	1.6	02/05/2025	Sarah Rushton, Karen Tricker	<p>Clarification that a molecular diagnosis refers to a diagnosis confirmed in the patient or their first degree relative.</p>

