**Treatment of Barth Syndrome by CARDIOlipin MANipulation (CARDIOMAN): A randomised placebo controlled pilot trial conducted by the nationally commissioned Barth Syndrome Service**

# CARDIOMAN

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***Details of Sponsor:***
University Hospitals Bristol NHS Foundation Trust
Research and Innovation
Level 3, Education & Research Centre
Upper Maudlin Street
Bristol BS2 8AE

Tel: 0117 342 0233
Fax: 0117 342 0239

***Chief Investigators & Research Team Contact Details***


Chief Investigator
**Professor Colin Graham Steward**

Professor Colin G Steward
School of Cellular & Molecular Medicine
Biomedical Sciences Building
University of Bristol
University Walk
Bristol, BS8 1TD

Tel: 0117 342 8044
Email: colin.steward@uhbristol.nhs.uk

Professor in Diabetes and Metabolic Endocrinology
**Professor Julian Paul Hamilton-Shield**

School of Clinical Sciences
Level 6
Education Centre
University Hospitals Bristol NHS Foundation Trust
Upper Maudlin Street
Bristol, BS2 8AE

Tel: 0117 342 0159
Fax: 0117 34 20178
Email: j.p.h.shield@bristol.ac.uk

Professor of Epidemiology
**Professor Andrew Robert Ness**

School of Oral & Dental Sciences
Biomedical Research Unit Offices
University Hospitals Bristol Education Centre
Lower Maudlin Street
Bristol BS1 2LY

Tel: (0117) 34 21751
Email: Andy.Ness@bristol.ac.uk

Senior Statistician
**Professor Chris Rogers**

Clinical Trials and Evaluation Unit
School of Clinical Sciences
University of Bristol
Level 7, Bristol Royal Infirmary
Upper Maudlin Street
Bristol BS2 8HW

Tel: 0117 342 2507

Email: chris.rogers@bristol.ac.uk

Chair of Clinical Chemistry
**Professor Simon Heales**

UCL Institute of Child Health
30 Guilford Street
London
WC1N 1EH

Tel: 0207 813 8321
Email: s.heales@ucl.ac.uk

Consultant Senior Lecturer
**Dr Chiara Bucciarelli-Ducci**

University Hospitals Bristol NHS Foundation Trust/University of Bristol
Level 7
Bristol Royal Infirmary
Upper Maudlin Street
Bristol
BS2 8HW

Tel: 0117 342 3287
Email: c.bucciarelli-ducci@bristol.ac.uk

Professor of Health Services Research
**Professor Barnaby Reeves**

Clinical Trials and Evaluation Unit
School of Clinical Sciences
University of Bristol
Level 7
Bristol Royal Infirmary
Upper Maudlin Street
Bristol
BS2 8HW

Tel: 0117 342 3143
Email: barney.reeves@bristol.ac.uk

Statistical advisor
**Ms Rosemary Greenwood**

Research Design Service
Education Centre
University Hospitals Bristol NHS Foundation Trust
Upper Maudlin Street
Bristol, BS2 8AE
Tel: 0117 342 0234
Email:
rosemary.greenwood@uhbristol.nhs.uk

Clinical Trial Coordinator

**Ms Lucy Dabner**
Clinical Trials and Evaluation Unit
School of Clinical Sciences
University of Bristol
Level 7
Bristol Royal Infirmary
Upper Maudlin Street
Bristol
BS2 8HW

Tel: 0117 342 2374
Email: lucy.dabner@bristol.ac.uk

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## Glossary / abbreviations

| | |
|---|---|
| ADR | Adverse drug reaction |
| AE | Adverse event - any undesirable event in a subject receiving treatment according to the protocol, including occurrences which are not necessarily caused by or related to administration of the research procedures. |
| AR | Adverse reaction – any undesirable experience that has happened a subject while taking a drug that is suspected to be caused by the drug or drugs |
| ATP | Adenosine 5' triphosphate – a multifunctional nucleotide that plays an important role in cell biology as a coenzyme involved in intracellular energy transfer |
| BDS | Bis die sumendum: twice daily |
| BEZA | Bezafibrate |
| BHI | Bristol Heart Institute |
| BNF | British National Formulary |
| BP | Blood pressure |
| BRI | Bristol Royal Infirmary |
| BRU | Biomedical Research Unit |
| BSF | Barth Syndrome Foundation |
| BSS | Barth Syndrome Service |
| BTHS | Barth syndrome |
| CK | Creatinine Kinase |
| CL | Cardiolipin |
| CRF | Case report form |
| CRIC | Clinical Research and Imaging Centre |
| CTEU | Clinical Trials and Evaluation Unit |
| DCM | Dilated cardiomyopathy |
| DMSC | Data monitoring and safety committee |
| EBV | Epstein-Barr virus |
| ECG | Graphical representation of electrical activity of the heart over time, as recorded by an electrocardiograph |
| ECMO | Extracorporeal membrane oxygenation |
| EF | Ejection Fraction |
| EME | Efficacy and Mechanisms Evaluation programme |
| EMEA | European Medicines Agency |
| FS | Fractional shortening |
| GCSF | Granulocyte colony stimulating factor |
| GOSH | Great Ormond Street Hospital |
| GP | General Practitioner |
| HDL | High density lipoprotein |

| | |
|---|---|
| HHb | Deoxyhaemoglobin |
| HMG CoA | 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors (Statins) |
| HR | Heart rate |
| HSG | Health Service Guidelines |
| ICH-GCP | International Conference for Harmonisation of Good Clinical Practice |
| IMP | Investigational medicinal product |
| LA | Left atrial |
| LDL | Low density lipoprotein |
| LFT | Liver function test |
| LV | Left ventricular |
| LVEF | Left ventricular ejection fraction |
| LVNC | Left ventricular non-compaction |
| MedRA | Medical Dictionary for Regulatory Activities |
| MHRA | Medicines and healthcare products regulatory agency |
| MLCL | Monolysocardiolipin |
| MRC | Medical Research Council |
| MRI | Magnetic resonance imaging |
| MRS | Magnetic resonance spectroscopy |
| NHS | National Health Service |
| NIHR | National Institute for Health Research |
| NIRS | Near infrared spectroscopy |
| NOS | Not otherwise specified |
| OD | Omne in die: once daily |
| PCr | Phosphocreatine |
| Peds QL™ | Paediatric quality of life inventory |
| PIL | Patient information leaflet |
| PKB | Patients Know Best website (https://www.patientsknowbest.com) |
| PPAR | Peroxisome proliferator-activated receptors |
| PW-TDI | Pulse Wave-Tissue Doppler Imaging |
| RA | Right atrial |
| RCT | Randomised controlled trial |
| REC | Research ethics committee |
| SAE | Serious adverse event - events which result in death, are life threatening, require hospitalisation or prolongation of hospitalisation, result in persistent or significant disability or incapacity. |
| SAR | Serious adverse reaction |
| SmPC | Summary of Product Characteristics |
| SOP | Standard operating procedure |
| SPC | Summary of Product Characteristics |
| SSAR | Suspected serious adverse reaction |
| SUSAR | Suspected unexpected serious adverse reaction - an untoward medical occurrence suspected to be related to a medicinal product that is not consistent with the applicable product information and is serious. |
| TDS | Ter die sumendum: three times a day |
| TSC | Trial steering committee |
| UH Bristol | University Hospitals Bristol NHS Foundation Trust |
| UK | United Kingdom |
| UoB | University of Bristol |
| USA | United States of America |
| $VO_2$ | Volume of oxygen |
| WBC | White blood cell count |

## 1. Trial summary

Barth syndrome is a rare, life threatening, genetic disease which affects young males. It is caused by abnormal fats (lipids) in the powerhouses of cells (mitochondria) and those who suffer with it often develop heart failure, heart rhythm abnormalities, bacterial infections, poor growth or feeding, weak muscles, developmental delay, severe exercise intolerance, lethargy and fatigue; all of which affect their daily life. Low white blood cell counts occur frequently due to intermittent or persistent reduction in numbers of the neutrophils that are responsible for fighting bacterial infections. This requires expensive and distressing injections to stimulate the bone marrow to produce more neutrophils. In addition, approximately one third of all males living with this disease in the UK have required heart transplantation.

Scientific research has shown that several treatments can improve the fat abnormalities in cells affected by Barth syndrome from either mice or humans; one of which is a drug called bezafibrate. Bezafibrate is particularly attractive as it has been safely administered for over 20 years in the UK to both adults and children for the treatment of high blood fats. The purpose of this study is to see if bezafibrate can be given safely and effectively to people with Barth syndrome in a blinded randomised trial.

Bezafibrate or an inactive (placebo) treatment will be given for 4 months, followed by a one month break, and then followed by 4 months of the alternate treatment (e.g. placebo if bezafibrate taken for the first 4 months and vice versa). Half of the participants will take bezafibrate first, the other half will take placebo first; participants and research staff will not know which order the treatments are given in (double-blind trial).

Once the participants have completed the first phase of treatment, the study statisticians will be unblinded to the treatment arms and will then assess whether or not there is evidence of efficacy of bezafibrate for the primary outcome.

Tests will be performed at commencement of the study and after each 4 month treatment period, looking for benefit in blood cells, exercise capacity, heart function or quality of life. The clinical studies of bezafibrate will run alongside laboratory work at University of Bristol and Great Ormond St Hospital to see the effect of bezafibrate on participants' cells and mitochondria (powerful electron microscopes show that these are abnormal in Barth syndrome). This is to see whether we can predict any improvement in symptoms in order to tell us which patients would benefit from this treatment in future. Laboratory work will also be carried out on participants' cells using another drug called resveratrol, which has also shown promise in laboratory tests, to see if this could provide an alternative treatment.

We wish to study up to 18 Barth syndrome boys and young men (all of those with Barth syndrome in the UK aged 6 years or over). We aim to use a national children's research network and/or local GPs to perform monthly blood tests in order to minimise the amount of travel to Bristol for participants. Safety will be monitored by an independent committee with the authority to recommend stoppage if obvious problems are seen.
If promising results are obtained with either or both drugs, we would share this information with American/European teams so that this work will have worldwide benefit. For this, we have the complete backing of our patients, their families and the respective UK and USA charities.

## 2. Background

Barth syndrome is a life threatening genetic disease which affects young males. It is caused by abnormal lipids in mitochondria. Those affected may be stillborn or develop heart failure during the first decade of life, suffer from bacterial infections, poor growth or feeding problems during childhood. Twenty six males and one female are currently alive with the disease in the UK. This disease carries many risks and problems for its sufferers, as well as major healthcare costs.

Boys and men living with the disease have severe exercise intolerance, lethargy and fatigue which affect their daily life; many use wheelchairs intermittently to aid mobility. Furthermore, children continue to die from this disease despite best conventional therapy including granulocyte colony stimulating factor (G-CSF) to stimulate neutrophil production, drugs to treat cardiomyopathy, and cardiac transplantation where required.

Scientific research has shown that several treatments can improve the fat abnormalities in cells affected by Barth syndrome in mice or humans [1]. One is a drug (bezafibrate) that has previously been used in children to lower blood fats as well as in adults with a muscle disease resulting from mitochondrial problems and the other is a food supplement (resveratrol). Both treatments have shown promising effects in a mouse that has heart problems like those seen in Barth syndrome [1].

There are no specific treatments for Barth syndrome other than supportive care provided for acute symptoms. Supplements such as coenzyme Q, carnitine and antioxidants are frequently used to treat other mitochondrial diseases but have proven ineffective in Barth syndrome [2] and there is no evidence that standard medications for cardiomyopathy ameliorate long-term outcome.

30% of living UK patients have undergone cardiac transplantation and several of these have died of cardiac rejection or post-transplant lymphoproliferative disease; others deteriorated too fast to undergo transplantation or died of complications of ventricular assist devices or ECMO whilst awaiting transplantation. In the past five years four babies/infants have been candidates for cardiac transplantation, requiring cumulatively approximately 20 months of intensive care bed stays. Sadly only one has undergone successful transplantation due to factors including shortage of donor hearts. Even those who have not received transplants can develop life-threatening heart rhythm disturbances.

Neutropenia is another life-threatening issue due to attendant serious bacterial infection. As a result, two thirds of UK patients require chronic subcutaneous injection therapy with granulocyte colony stimulating factor (G-CSF), a distressing and expensive medication. Furthermore, the management of neutropenia in this disease is challenging since patients have highly variable neutrophil counts, preventing administration of a consistent daily dose and requiring repeated blood counts and physician oversight. They often remain intermittently neutropaenic even when receiving G-CSF.

Lethargy and fatigue are universal, interfering with schoolwork and play and often necessitating the use of wheelchairs. Similarly patients cannot hold down strenuous or demanding jobs. Many patients have major feeding problems from the neonatal period through into adult life and require long term supplemental feeding via gastrostomies.

There can be rapid deterioration during periods of stable health and when under expert medical care. Ventricular arrhythmia (tachycardia or fibrillation) affects 10% of adolescents and can

cause sudden cardiac death at any stage of childhood, including the neonatal period [3]. These seemingly random acute crises are not predictable by genotype or recent medical history, producing a need for cardiac resuscitation training and chronic anxiety in affected families.

It is clear that more disease-specific therapy is required to prevent morbidity, mortality, psychological distress and disruption of quality of life, potentially producing major savings for the NHS. We in the UK are uniquely well-positioned to explore such therapy, having the world's highest density of diagnosed patients and the only national multidisciplinary service. The NHS Specialised Services Barth Syndrome Service currently cares for 26 living boys and one girl from England, Scotland and Wales (from approximately 200 diagnosed worldwide), of whom 18 are above six years of age and candidates for the trial proposed.

Several of the laboratory and clinical readouts proposed in this study (cardiolipin profiling, cardiac MRS and electron microscopic evaluation of mitochondrial morphology) could have importance for assessing genotype/phenotype correlation and future therapies in Barth syndrome beyond the scope of this randomised trial. Patients with unexplained conditions very closely allied to Barth syndrome are known to this team. The technical expertise developed here may allow better investigation of these patients and those with other unexplained conditions such as idiopathic neutropenia or cardiomyopathy.

'Gold standard' evidence from a randomised controlled trial is now required to investigate the potential risks and benefits of bezafibrate treatment in this population. Prepublication data from Dr Ren *et al* at New York University (2012) demonstrated clear effects of bezafibrate and resveratrol in partially normalising the highly skewed cardiolipin (CL) ratio that uniquely characterises humans with the disease and Barth mouse knockdown cells [1]. Bezafibrate has since been shown to improve ventricular function at supraphysiological doses in a knockdown mouse model of the disease. This was shown both in cardiomyopathy induced by adrenergic (isoproterenol) stress and by chronic administration in unstressed animals. In the latter it prevented the cardiac deterioration typically seen by 7 months of age [4]. Surprisingly, in contradiction to the previous cellular evidence, this cardiac benefit was not accompanied by improvement of the CL ratio; it is postulated that the drug works via increasing mitochondrial biogenesis (mitochondrial numbers) in cardiomyocytes via a role in activating peroxisome proliferator-activated receptors (PPARs) rather than ameliorating the abnormal lipid chemistry. Bezafibrate has also been shown during a 4-month period at a clinically relevant dose to protect cardiac left ventricular systolic function and, in combination with everyday voluntary running, to significantly ameliorate the impaired exercise capacity in TazKD mice [5].

Bezafibrate is an European Medicines Agency (EMEA) approved lipid-lowering drug with established use in adults and children and a good safety record in long-term use [6]. The BNF recommends doses of 200mg OD to TDS for 10-18 year olds, and Bezafibrate use is well tolerated in younger children in the dose range 5-20 mg/kg/day [7-9] and in adults up to 600-900mg/day [10]. Bezafibrate has been reported to significantly ameliorate a mitochondrial myopathy (CPT2 deficiency) in adults [11] as well as giving promising results in a range of animal/human cellular models of mitochondrial disease.

Resveratrol, a naturally occurring food supplement available from nutraceutical companies, affects energy metabolism and mitochondrial function. Previous human clinical trial data is limited, with mild gastrointestinal symptoms occurring at doses above 1g/day [12]. In a 2011 double-blind crossover trial in healthy obese men resveratrol (at 150mg/day for 30 days) produced a range of statistically significant beneficial clinical effects [13]. Resveratrol has been included for comparative *in vitro* studies because although bezafibrate and resveratrol had

equivalent effects in the mouse model/cells, it had the significantly more convincing effect on improving CL ratio in human Barth syndrome fibroblasts. However, it will not be used in this trial due to NIHR EME Board concerns that its use could aggravate pre-existing neutropenia and that half-life in blood is extremely short.

Barth syndrome is characterised by many private gene mutations; more than 120 have been identified in only 200 affected persons [2]. There is profound phenotypic variability between affected patients within families and those in different families, both in severity of cardinal problems (e.g. cardiomyopathy, neutropenia) and the number of different organs that are significantly affected. There has been no definitive proof of any genotype/phenotype correlation in Barth syndrome.

## 3. Aims and objectives

We hypothesise that bezafibrate (and/or resveratrol in-vitro) will increase mitochondrial biogenesis and potentially modify the cellular ratio of monolysocardiolipin to L4- cardiolipin, ameliorating disease phenotype in those living with the disease, providing that the drug is free of significant side-effects at clinically effective doses.

The major questions to be answered are as follows:

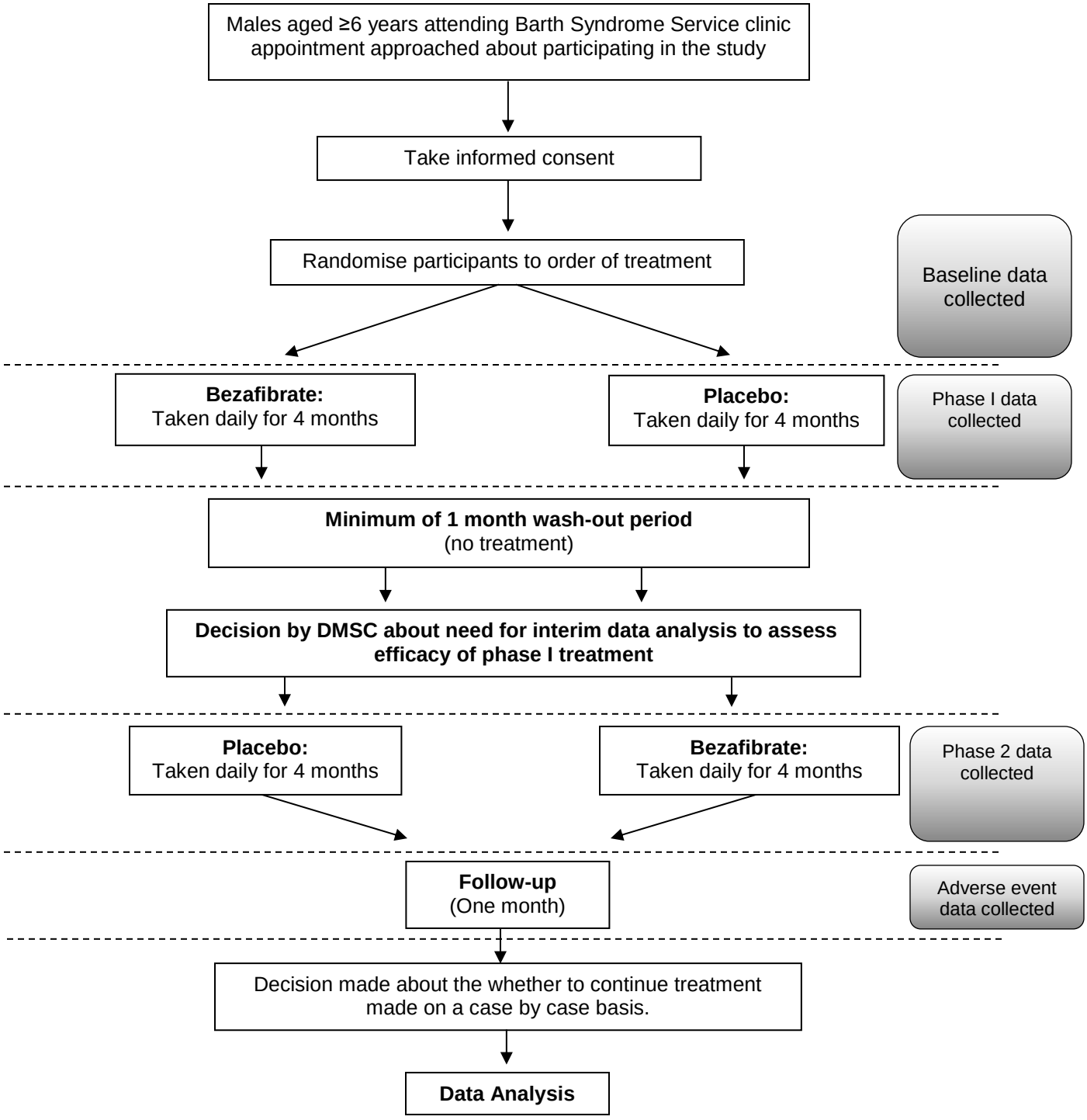
1. What is the effect on biochemical/clinical outcome measures and quality of life of bezafibrate treatment in comparison to placebo in Barth syndrome?
2. Is it possible to correlate clinical improvements with *in vitro* analysis of cardiolipin ratio/profile and mitochondrial morphology in each participant's cells when exposed to bezafibrate in laboratory culture? How does this contrast with an *in vitro* response to resveratrol exposure? This could indicate whether a trial examining the efficacy of resveratrol may be required in the future to determine the optimal choice of drug.
3. What are the most feasible methods and standardised outcome measures that may allow better conduct of future trials (e.g. larger multinational trials) and evaluations in Barth syndrome?
4. Is it possible to create a research infrastructure which optimises recruitment, retention and communication with families and people with Barth syndrome?
5. What are the participant and family perceptions of research and any important potential barriers to participation?

The primary outcome to be measured is whole body oxygen consumption during peak bike ergometry exercise (i.e. peak $VO_2$). Improved quality of life is an overarching goal, principally through improved muscle function and is an important secondary outcome. A broad range of biochemical, mitochondrial and clinical readouts has also been selected as secondary outcome measures (see section 4.6) including cardiolipin ratio on which this work is predicated.

This study would also aim to look at many aspects of the practicality and robustness of study design.

## 4. Plan of Investigation

### 4.1 Study schema



## 4.2 Trial design

This study is a double-blinded, randomised, placebo-controlled crossover study. Treatment will be given in two 4 month long phases with a minimum of one month washout period between these where no treatment is given. Participants will be followed up as part of the study for one month after the end of second treatment period.

After phase 1 treatment has been completed by participants, a decision will be made by the Data Monitoring and Safety Committee as to whether an interim analysis should be conducted to assess the efficacy of phase I treatment. If so, confidential un-blinding will be performed by the study statistician to assess the response to bezafibrate. If an improved peak $VO_2$ (primary outcome) is demonstrated in ≥ 50% of participants receiving bezafibrate, phase 2 of the study will commence as planned. If no response is demonstrated, or a response is demonstrated in less than 50% of participants receiving bezafibrate, phase 2 of the study will not go ahead and the study will finish.

## 4.3 Key design features to minimise bias

**(a) Confounding/allocation bias** (systematic differences between baseline characteristics of the groups that are compared)

Participants will act as their own controls providing that they complete both phases of the trial, precluding confounding. The allocation sequence (order of drug and placebo in the two phases) will be randomised and concealed making prediction of the next treatment allocation difficult, minimising confounding for any order effect; this will also minimise confounding of the treatment effect for phase 1 if phase 2 proves not to be feasible. However, the small numbers of participants available for this study may introduce imbalances between the groups by chance (see section 5.1).

**(b) Performance bias** (systematic differences between groups in the care that is provided, or in exposure to factors other than the interventions of interest)

This bias will be minimised by:

- Blinding of all participants and personnel (apart from the dispensing pharmacist) and the success of blinding will be assessed (participants may become un-blinded due to side effects or marked symptomatic improvement);
- Administering the intervention, comparator and other procedures undertaken during the trial according to standard protocols;
- Pre-defining all procedures for participant follow-up and applying the procedures to all participants in the same way;
- Monitoring adherence to the protocol.

**(c) Detection bias** (systematic differences between groups in how outcomes are determined)

This bias will be minimised by:

- Blinding of all individuals assessing outcomes, including cardiologists/technicians conducting the bicycle ergometry tests (i.e. peak $VO_2$), and participants and assessing the success of blinding;

- Using an objective primary outcome measure of peak whole body oxygen consumption on bicycle ergometry which is a well validated indicator of skeletal muscle mitochondrial dysfunction in Barth syndrome.

**(d) Attrition bias** (systematic differences between groups in withdrawals from a study)

This bias will be minimised by:

- Maintaining contact with participating families throughout the duration of the trial to maximise the proportion of participants for whom all outcome data are available (see section 5.12), even if the drug or placebo intervention has to be stopped for a participant (this may be facilitated through the follow-up of patients through the Barth Syndrome Service if necessary), and the proportion of participants who receive the intervention to which they were allocated.
- Implementing measures to promote adherence to random allocations.
- Pre-specifying analysis of phase 1 only if a proportion of participants (to be specified) fail to complete phase 2.
- Using intention to treat analysis.

**(e) Reporting bias**

This type of bias will be minimised by having pre-specified outcomes (see section 4.6) and a pre-specified analysis plan (see section 6.0).

### 4.4 Trial population

The reference population is males with a confirmed diagnosis of Barth syndrome.

The study population is males aged ≥6 years in the UK, with a confirmed diagnosis of Barth syndrome with the following inclusion/exclusion criteria applied. Males under 6 years of age are not included in this patient population because of difficulties in obtaining data on the primary outcome through bicycle ergometry in young children.

#### 4.4.1 Inclusion criteria

Participant may enter study if ALL of the following apply:

1. Male aged ≥6 years old
2. Clinical diagnosis of Barth syndrome with characteristic abnormality of the L4-cardiolipin/monolysocardiolipin ratio plus identified mutation in the tafazzin gene
3. Under the care of the NHS Barth Syndrome Service (BSS)
4. Stable cardiac condition
5. Able to swallow bezafibrate tablets (similar size to ibuprofen tablets)

#### 4.4.2 Exclusion criteria

Participant may not enter study if ANY of the following apply:

1. Known hypersensitivity to bezafibrate, to any component of the product or to other fibrates
2. Known photoallergic or phototoxic reactions to fibrates.
3. Hepatic dysfunction and/or liver function tests greater than 2x normal

4. A shortening fraction of <25 (or a significant drop in shortening fraction in the previous year)
5. Documented atrial or ventricular arrhythmia (atrial/ventricular tachycardia or atrial/ventricular fibrillation) that has not been stabilised with treatment.
6. Renal impairment (creatinine clearance < 90 mL/min)
7. Pre-existing known gallbladder disease.
8. Recent unspecified significant deterioration in general health
9. Prisoners and adults lacking capacity to provide informed consent

There are reports of rhabdomyolysis occurring in patients treated with a combination of bezafibrate and statins. Four of the five males in this patient cohort who have undergone cardiac transplantation are maintained on the statin pravastatin. This has been discussed with the cardiology team at Great Ormond Street Hospital who are agreeable for this medication to be ceased during the trial period.

### 4.5 Trial interventions

Bezafibrate is classed as an Investigational Medicinal Product (IMP) and is therefore under the regulation of the Medicines and Healthcare products Regulatory Agency (MHRA).

All participants will receive 4 calendar months of the intervention (bezafibrate) **and** 4 calendar months of the placebo (if the trial proceeds to phase 2. See section 4.2 for details). The order in which they will be administered will depend on the allocated treatment arm at first randomisation. Those first allocated to the intervention at randomisation will be given bezafibrate followed by the placebo. Those first allocated to the placebo at randomisation will be given the placebo followed by bezafibrate. Both arms will have a minimum of 1 calendar month washout period between the intervention and placebo administered, where no treatment is given.

Participants will be dispensed at least a month's supply of either bezafibrate or placebo at any one time. For participants who live some distance away from Bristol, they will receive further dispensed medications via courier.

The study intervention will be prescribed once at the start of the study. As investigators and participants are blinded, the prescription will not specify which medication (bezafibrate or placebo) will be taken first: this will be determined by the randomisation list held by the UH Bristol Clinical Trials Pharmacy department. All dispensing will be managed by UH Bristol Clinical Trials Pharmacy Department, including cross-over to the other study medication if the study proceeds to the second phase

**Intervention:** Bezafibrate taken orally in tablet formulation.

- Children aged 6-9 years: Commence on 100mg OD for the first month and if well tolerated increase to 100mg BD for the remaining 3 month treatment period
- Children aged 10-17 years: commence on 200mg OD for the first month and if well tolerated increase to 200mg BD for the remaining 3 month treatment period
- Adults (≥ 18 years): 200mg BD

**Placebo:** Tablet formulation with no active substance taken orally. The placebo will look, taste and smell as similar as possible to the intervention.

Bezafibrate and placebo will be stored, packaged and labelled by UH Bristol Pharmacy Department. Drug accountability will also be managed and monitored by UH Bristol Pharmacy Department. Both will be performed according to their SOPs, which are in accordance with GCP.

Compliance with study treatment will be assessed by asking participants to return any unused medications.

**Concomitant medication** (including contraindicated medication/treatments) [14]:

- Care is required in administering bezafibrate to patients taking coumarin-type anti-coagulants, the action of which may be potentiated. The dosage of anti-coagulant should be reduced by up to 50% and readjusted by monitoring blood coagulation.
- As bezafibrate improves glucose utilisation the action of antidiabetic medication, including insulin, may be potentiated and should be considered.
- Should combined therapy with an ion-exchange resin be considered necessary, there should be an interval of 2 hours between the intake of the resin and bezafibrate, otherwise the absorption of bezafibrate may be impaired.
- In isolated cases, a pronounced though reversible impairment of renal function (accompanied by a corresponding increase in serum creatinine level) has been reported in organ transplant patients receiving immuno-suppressant therapy and concomitant bezafibrate. Accordingly, renal function should be closely monitored in these patients and, in the event of relevant significant changes in laboratory parameters, bezafibrate, should if necessary, be discontinued. (NB creatinine will be measured at 2 weeks after commencing study medication in both phases of the study in participants who have received an organ transplant.)
- MAO-inhibitors (with hepatotoxic potential) should not be administered together with bezafibrate.
- Interaction between HMG CoA reductase inhibitors and fibrates may vary in nature and intensity depending on the combination of the administered drugs. A pharmacodynamic interaction between these two classes of drugs may, in some cases, also contribute to an increase in the risk of myopathy for specific dose recommendations of statins. Refer also to the SPC of the relevant product.

**Granulocyte colony-stimulating factor (GCSF)**: Participants will continue to be prescribed GSCF as clinically indicated as there are currently no known drug interactions with this treatment.

***Blood count deterioration:*** if a patient is shown to have a neutrophil count below 0.5 x $10^9$/L, either in their monthly monitoring counts or if a participant presents with sign and symptoms of infection and requires additional blood tests as part of their normal care, GCSF therapy will be initiated or dose/frequency of dosing reviewed and possibly increased in line with routine care of these patients. Subsequent weekly full blood counts will be requested on post G-CSF days for the next two weeks (as this is the routine way of monitoring neutrophil counts in these patients). If the patient has three consecutive blood counts of less than 0.5 x $10^9$/L, administration of the intervention will be terminated.

**Post-trial treatment:** Once the participants have completed the study treatment their medical care will revert to the standard care received from the Barth Syndrome Service. However, if bezafibrate is shown to have a beneficial effect on individual participants, provision will be made for them to continue receiving bezafibrate, on a case-by-case basis.

**Current licensed indications:**

- Treatment of severe hypertriglyceridaemia with or without low HDL cholesterol.
- Mixed hyperlipidaemia when a statin is contraindicated or not tolerated.

**Elimination half-life of drug in the body:** 1-2 hours

For further information about bezafibrate please see the Investigational Medicinal Product Dossier Bezafibrate 100mg tablets and placebo Bezafibrate 100mg tablets (Version 1.0, 14/02/2018).

## 4.6 Primary and secondary outcomes

### 4.6.1 Primary outcome

The primary outcome measure is peak oxygen consumption on bicycle ergometry (i.e. peak $VO_2$), because this outcome is strongly associated with activity intolerance and may well correlate with subjective fatigability which we believe to be the most important determinant of quality of life in these patients. Peak $VO_2$ will be assessed at baseline and in the final week of each treatment phase.

### 4.6.2 Secondary outcomes

1. Monolysocardiolipin/tetralinoleoyl-cardiolipin MLCL/L4-CL ratio/cardiolipin profile in blood cells
2. PCr/ATP ratio in cardiac muscle on 31P Magnetic Resonance Spectroscopy
3. Skeletal muscle oxidative function/ on 31P Magnetic Resonance Spectroscopy
4. Quality of life (QoL) assessed using age-appropriate PedsQL questionnaires
5. Absolute neutrophil count
6. Amino acid expression (serum arginine and cysteine levels)
7. Cardiac function (LVEF and shortening fraction)
8. Mitochondrial size in lymphocytes
9. Numbers of mitochondria (per lymphocyte)
10. Total area of mitochondria per lymphocyte
11. Area of mitochondria as proportion of cytoplasm
12. Mitochondria function and cristae organisation in lymphocytes
13. Arrhythmia profile from 12 lead ECG at rest and during exercise (for potential rhythm abnormalities)

See Section 5.6 Data Collection for the timing of these assessments.

The work around the immortalised cell lines does not form part of this protocol and will be described in a separate protocol.

In addition to the data collected from the clinical trial we propose to integrate qualitative research methods in order to explore patients' and families' experiences of the different interventions. Parents of younger patients (<18 yrs) and patients (>14yrs, suitability for interviewing to be determined on advice from the Clinical Psychologist known to the patient) will be invited to take part in semi-structured one-to-one interviews in the assessment periods after the first and second interventions. The first interview will last approximately 20 minutes and explore the parents'/participant's experience of the intervention. The second interview will last

approximately 40 minutes and will explore the parents'/participant's experience of the second intervention and their perception of participating in the trial as a whole. Interviews with patients and parents will be digitally recorded with their consent and transcribed verbatim. Data from interviews will be analysed using framework analysis methodology [15] with the aid of the NVivo software package.

### 4.7 Sample size calculation

This is a single centre study to be performed in patients attending the NHS National Barth Syndrome Service at UH Bristol. A total of 18 males aged between six and 24 years currently attend the service. We anticipate that between 12 and 15 UK patients are likely to consent to take part.

In a simple crossover trial (a single intervention and control comparison), the standard deviation (SD) of the within subject difference between treatments is given as: sqrt (2 x within subject SD) [16]. In a crossover trial, all participants constitute their own control.

In this trial, the difference in mean peak volume of $O_2$ consumption between placebo and bezafibrate phases will be tested, assuming a 2-tailed alpha of 0.05. For a sample size of 12 participants, the trial will be able to detect a difference of 0.90 (within subject) SDs with 80% power, or 1.05 SDs with 90% power.

## 5. Trial methods

### 5.1 Description of randomisation

The random sequence allocations of bezafibrate or placebo will be generated prior to the start of the study by a CTEU statistician, using blocks of undisclosed size. The sequence will then be 'attached' to a list of consecutive study IDs and provided to the clinical trials pharmacy department.

The allocations of drug or placebo in the second phase of the trial will be the opposite of the first phase. Once a participant has given consent and eligibility has been documented and confirmed, they will be allocated to the next consecutive study ID. A doctor will prescribe the study 'medication' and the pharmacy will dispense the appropriate intervention according to the participant's study ID and phase in the trial.

Allocation concealment will minimise selection bias. The sequence of random allocations will be generated by computer and will be concealed from all clinical and research personnel.

### 5.2 Blinding and code breaking

This study is double blinded: neither the participants nor investigators (or any other member of the research team apart from the pharmacist) will have knowledge of the allocated treatment being given, or the order in which it is given.

The tablets containing bezafibrate or placebo do not have a particularly strong or unusual smell or taste, so we do not anticipate un-blinding will occur due to the characteristics of the drug. The tablets for active drug and placebo will look identical, which should help to minimise the un-blinding of participants and subsequently investigators. We are aware that bezafibrate may

induce side-effects in some patients that will inadvertently un-blind participants and we acknowledge this may be a limitation of this study.

The patient information leaflet and the process of informed consent will explain the uncertainty around the potential beneficial effects of bezafibrate over a placebo. Therefore, in the event of inadvertent un-blinding of a participant, the participant should not have a strong expectation that one or other method should lead to a more favourable result.

Participants will be made aware before entering the study that they will not be told which treatment they will receive in each phase. Doctors will prescribe the 'study medication' rather than specifically bezafibrate or placebo and the prescription for the 'study medication' will be sent to pharmacy, who will then provide the medication as specified according to the pre-determined list drawn up by the study statistician prior to recruitment (as described earlier). The allocations will only be known by pharmacy and the study statistician and will not be disclosed to any other member of the research team.

Members of the health care team can request un-blinding of the study medication in either phase in the event of a serious adverse event (SAE), if they consider that this information will alter their management of the SAE. During office hours CTEU Bristol should be contacted for requests to unblind. Out of office hours UH Bristol Pharmacy Department should be contacted using their on-call system and a request made to contact a member of the Clinical Trials Pharmacy team. The on call pharmacist will have access to the un-blinding list. All instances of un-blinding will be documented, including who requested the un-blinding, why it was required, the time and date and who performed the un-blinding.

### 5.3 Research procedures

Detailed assessments will be performed in Bristol at baseline and at the end of each treatment phase. These assessments will be timed during the final week of therapy so that patients are still receiving interventions/placebo at the time of testing but will have had maximum cumulative exposure to the drug or placebo. These will include:

- Anthropometric data (height and weight)
- Medical history and examination (including resting blood pressure, heart rate and oxygen saturation)
- Trans-thoracic echocardiographic determination of cardiac function at rest and during exercise (stress Echo) (see Appendix 1).
- Tissue Doppler studies (see Appendix 1).
- Modified McMaster exercise protocol to assess peak oxygen consumption using an electronically braked GE Healthcare exercise echocardiography
- Muscle deoxygenation (HHb) during exercise using near infrared spectroscopy (NIRS) over the vastus lateralis muscle.
- Ventricle volumetrics using cardiac Magnetic Resonance Imaging (MRI)
- PCr/ATP ratio obtained from Magnetic Resonance Spectroscopy
- Quality of Life questionnaires (PedsQl)
- Qualitative assessments (semi-structured one-to-one interviews) to assess patient experiences of the intervention and participating in a trial as a whole, along with a dietary evaluation
- Cardiac arrhythmia profile from 12 lead ECG at rest and during exercise

In those who can tolerate an MRI scan lasting approximately one hour without general anaesthetic, we will use 31P magnetic resonance spectroscopy (performed on a Siemens 3T Magnetom Skyra MRI scanner) to assess phosphocreatine/adenosine triphosphate ratio (PCr/ATP) in myocardium and oxidative function (PCr recovery kinetics) in skeletal muscle. Cardiac MRI imaging will assess ventricle volumetrics. Detailed MRI/S methods will be documented in a separate protocol.

Imaging tests will be performed at the Clinical Research Imaging Centre (CRIC, a joint UoB/UHBristol facility).

A total of 20 mL of blood will be taken at each assessment visit for the following tests:

1. At baseline only: establishment of an EBV transformed lymphoblast line* for *in vitro* incubation with resveratrol or bezafibrate and assessment of cardiolipin ratio.
2. At baseline and end-of-treatment assessments: Full blood count, absolute neutrophil count, urea/electrolytes, liver function tests, creatinine kinase, plasma arginine/cysteine, full lipid profile (total cholesterol, high density lipoprotein, triglycerides), brain natriuretic peptide (as a blood marker of LV function).
3. Mitochondrial tests: assessment of detailed cardiolipin profiling (to be performed at Great Ormond Street Hospital) and calculation of MLCL/L4-CL ratio. Mitochondria of the blood cells will be examined by electron microscopy of lymphocytes and measurements of size, number and shape recorded. This work will be performed at the University of Bristol. Lymphocytes extracted from 5 mL whole blood will undergo analysis for mitochondrial function studies including analysis of respiratory chain enzyme complexes using established spectrophotometric methods.

* For patients who have an existing EBV cell line available from a separate study entitled ‘Cardiolipin metabolism and mitochondrial dysfunction in the pathogenesis of Barth syndrome’, consent will be sought to use their existing cell line, rather than establish a new one.

Informed consent will be sought from the patients and/or their families to store and use the EBV transformed lymphoblast lines (also known as ‘immortalised’ cells) for future research.

Subjective quality-of-life will be assessed using age appropriate Peds QLTM Paediatric Quality of Life Inventory assessment forms and parental questionnaires. These include forms suitable for young adults (18-25).

### 5.4 Duration of treatment period

The duration of the treatment period will be for a minimum of 9 months in total: 4 months of bezafibrate followed by 4 months of placebo (or vice versa), with a minimum washout period of one month in between, where no treatment is given between the two treatments.

If the DMSC deem it necessary for an interim analysis after the first phase of treatment and no effect of bezafibrate is found, the study may not continue on to the second phase. If this happens, the duration of treatment will be four months.

## 5.5 Definition of end of trial

The end of the trial for participants will be after they have completed the treatment period and a one month follow-up period (or have been lost to follow-up or died).
The end of the trial as a whole will be when all recruited participants have completed the treatment and follow-up period ***and*** all data has been collected, data queries answered and all laboratory samples have been processed and analysed.

## 5.6 Data collection

Data collection will occur at four different time-points: baseline, end of treatment phase 1, end of treatment phase 2 and after completion of one month follow-up.

A schedule of investigations and assessments over the 10 months of the trial is shown below.

**Table 1 Schedule of Events**

| ***Event*** | | ***Baseline*** | ***Phase 1*** | | | | ***Washout Period*** | ***Phase 2*** | | | | ***Follow-up*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Month*** | | ***0*** | ***1*** | ***2*** | ***3*** | ***4*** | ***5*** | ***6*** | ***7*** | ***8*** | ***9*** | ***10*** |
| *Informed Consent* | | ✓ | | | | | | | | | | |
| *Height & weight* | | ✓ | | | | ✓ | | | | | ✓ | |
| *Medical history* | | ✓ | | | | | | | | | | |
| *Clinical examination*[+] | | ✓ | | | | ✓ | | | | | ✓ | |
| *Bicycle ergometry (exercise bike test)* | *Peak oxygen consumption* | ✓ | | | | ✓ | | | | | ✓ | |
| | *Muscle deoxygenation (HHb) (NIRS)* | ✓ | | | | ✓ | | | | | ✓ | |
| | *Tissue Doppler studies* | ✓ | | | | ✓ | | | | | ✓ | |
| *Echocardiogram (at rest and during exercise)** | | ✓ | | | | ✓ | | | | | ✓ | |
| *12 Lead ECG at rest and during exercise (during echo)* | | ✓ | | | | ✓ | | | | | ✓ | |
| *Blood sample (20-30 ml total)*[#] | *Transformed lymphoblast line for in vitro incubation with Bezafibrate and Resveratrol* | ✓ | | | | | | | | | | |
| | FBC, absolute neutrophil count, urea/electrolytes, LFTs, CK, plasma arginine/ cysteine, full lipid profile (total cholesterol, high density lipoprotein, triglycerides), brain natriuretic peptide | ✓ | | | | ✓ | | | | | ✓ | |
| | *Mitochondrial assessment*[#] | ✓ | | | | ✓ | | | | | ✓ | |
| *Blood sample (safety assessment) done locally to patient* | FBC including absolute neutrophil count, routine renal and liver function tests, plasma triglyceride/total cholesterol/LDL- | | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | | |

| ***Event*** | | ***Baseline*** | ***Phase 1*** | | | | ***Washout Period*** | ***Phase 2*** | | | | ***Follow-up*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | cholesterol and Creatine Kinase, Creatinine** | | | | | | | | | | | |
| ***Month*** | | ***0*** | ***1*** | ***2*** | ***3*** | ***4*** | ***5*** | ***6*** | ***7*** | ***8*** | ***9*** | ***10*** |
| *Cardiac/skeletal muscle MRI/MRS scan$* | | ✓ | | | | ✓ | | | | | ✓ | |
| *QoL: /Paeds QoL* | | ✓ | | | | ✓ | | | | | ✓ | |
| *Adverse events* | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| *Drug prescribing* | | ✓ | | | | | | | | | | |
| *Drug dispensing* | | ✓ | | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | |
| *Qualitative Interview* | | | | | | ✓ *(20 mins)* | | | | | ✓ *(40 mins)* | |

+ Including resting blood pressure, heart rate and oxygen saturation
* Cardiac status: LVEF and shortening fraction
# Samples for detailed cardiolipin profiling and calculation of MLCL/L4-CL ratio; examination of mitochondria of the blood cells by electron microscopy and measurements of size, number and shape recorded; analysis of mitochondrial function studies including analysis of respiratory chain enzyme complexes.

**to be measured at 2 weeks after initiated study drug in both phases for participants who have had an organ transplant

$ Cardiac MRI will be used to assess:

- Right and left ventricle volumetrics.

MRS will be used to assess:

- Phosphocreatine/adenosine triphosphate ratio (PCr/ATP) in myocardium and oxidative function
- ATP production in skeletal muscle.

Monthly blood tests will be performed throughout the study at the patient's local hospital or GP surgery to assess the continuing safety of the bezafibrate in this population. An additional blood test for creatinine will be carried out at 2 weeks after the initiation of study drug in both phases in participants who had had an organ transplant. Absolute neutrophil count, routine renal and liver function tests, plasma triglycerides/total cholesterol/LDL-cholesterol and creatine phosphokinase will be tested. The study research nurses will facilitate the arrangement of these tests with the patients/families and their local provider and will obtain the results from the place of testing for safety monitoring.

### 5.7 Source data

The primary data source will be the participant's medical notes for clinical data, including peak oxygen consumption (primary outcome), cardiac function parameters and NIRS data. The laboratory reports will be the primary data source for the results of the blood tests, whilst records from laboratory books, images, print-outs and electronic data will form the source data for mitochondrial structure and function.

The MRI/MRS outputs with associated reports will form the source data for imaging tests such as MRS and stress echocardiogram. The completed patient questionnaires will be the primary data source for these measures.

Source data for some adverse events will be recorded on the 'Patients Know Best' web-based service that some of the Barth syndrome patients already use. This is an NHS-contracted certified private IT provider of a patient information site to allow patients and medical carers easy online access to their personal medical information and treatment plans.

### 5.8 Planned recruitment rate

Eligible patients will be recruited from the national clinics held in Bristol. All patients in the UK with Barth syndrome use our national specialist multi-disciplinary service and we expect to recruit approximately 12-15 of the 18 eligible patients, from several clinic dates over a short period of time (expected to be approximately 2-4 weeks).

### 5.9 Participant recruitment

Potential participants will be identified through the Barth Syndrome Service, which is a centralised NHS Specialist Service run in Bristol. Potential recruits are already well known to the research team due to the small numbers with the disease. The research team is led by Professor Colin Steward, who liaises with all members of the NHS Barth Syndrome Service via monthly multidisciplinary team meetings.

An invitation letter and Participant Information Leaflet (PIL) will be sent to the participant, or their parents/guardians, well in advance of their clinic visit (likely to be at least once month) to provide enough time for the participant or their families to read and digest the information about the study.

In order to make arrangements for when participants and their families attend the clinic in Bristol we will need some prior indication as to whether the patient is likely to participate in the study. Therefore we will contact the patient or their family by telephone before their clinic appointment

to determine if they are interested in participating on the understanding that they may change their minds.

Patients will be approached to participate in the clinic appointment by Professor Colin Steward and/or a research nurse who will discuss the study in detail and will answer any questions that patients or their families may have about the study. If the patient and/or the family is willing, informed consent will be taken during this clinic visit.

### 5.10 Discontinuation/withdrawal of participants

Each participant has the right to withdraw at any time by informing any member of the research team and without needing to give a reason. In addition, the investigator may withdraw the participant from their allocated treatment arm if they do not feel it is in the best interest of the participant to continue with the treatment due to adverse side-effects.

If a participant wishes to withdraw, we will continue to analyse any data already collected, unless the participant expresses a wish for their samples and any associated data to be destroyed. We will not collect further data on them after the time of withdrawal but will monitor adverse events for reporting where necessary.

Participants will be followed-up for one month after withdrawal of treatment for assessment of safety. A withdrawal form will be completed at the time of the withdrawal, documenting the reason for the withdrawal (if known) and the wishes of the participant with respect to their data.

### 5.11 Frequency and duration of follow up

Follow up for this study will continue for one month after the study treatment has ended in order to assess the safety of bezafibrate in this patient population.

Monthly blood tests will be performed throughout the study to assess full blood count including absolute neutrophil count, routine renal and liver function tests, plasma triglyceride/total cholesterol/LDL-cholesterol and creatinine kinase. The latter is included since bezafibrate can induce rhabdomyolysis, although this risk is most significant in those being additionally treated with statins [17]. An additional blood test for creatinine will be carried out at 2 weeks after the initiation of study drug in both phases in participants who had had an organ transplant. Where patients are receiving GCSF for management of severe neutropenia, these tests will follow our usual testing protocol and be performed on the day following GCSF administration. This method of assessment is used since some patients will show unpredictably exuberant responses to GCSF, which is best detected using this method in order to allow dose manipulation by monitoring of "peak" rather than "trough" counts.

These safety monitoring blood tests will be performed at a GP surgery/hospital local to the participant, or an appropriate assessment visit to Bristol.

The study research nurses will have regular contact with participants to collect adverse event data: after the first week of starting treatment and then monthly thereafter until end of follow-up. Patients will be asked to report (by telephone or via the Patients Know Best web service which the Barth Syndrome Service utilise for secure electronic communication with patients / parents / guardians) any unexpected symptoms or hospital visits/admissions to the Barth Syndrome Service clinical and nursing team, or the research team. If participants require help out of

normal service hours, they may contact the on-call haematology registrar or Consultant at Bristol Royal Hospital for Children who will be able to contact one of the clinicians allied to the Barth Syndrome Service.

### 5.12 Likely rate of loss to follow-up

As all of the potential participants are already well known to the research team and would use the Barth Syndrome Service outside of this study, we do not anticipate there to be any loss to follow-up. Therefore there are no special features to minimise attrition bias. Established CTEU methods will be used to maximise the proportion of participants for whom all outcome data are available and the proportion of participants who receive the intervention to which they were allocated.

### 5.13 Expenses

Participants and their families will be reimbursed for their travel expenses to and from Bristol, plus 1-2 overnight stays for participants who live further away.

## 6. Statistical analyses

### 6.1 Plan of analysis

It is hoped that all participants will complete and contribute data to both phases of the crossover study, although we recognise that some missing data are likely due to the fragile nature of Barth syndrome.

We aim to retain all participants in the trial for the full duration of 10 months follow-up, irrespective of any periods when an intervention has to be suspended because of ill health. The only exceptions will be when a participant expressly requests to end all aspects of their participation in the trial or cannot continue because of deteriorating health. We propose that primary analyses should respect the intention-to-treat principle but envisage that additional secondary or sensitivity analyses may be specified in a detailed analysis plan, written prior to completing the final data queries for final analyses.

Our plan for analyses assumes that the continuously scaled outcome data (or transformed data) will be distributed satisfactorily to allow parametric methods to be applied. We propose to analyse the data using mixed effects regression methods that will allow for participants' data to be included if not complete. The regression models will estimate effects for both the treatment factor (2 levels) and time period of intervention (2 levels), adjusting for baseline assessments of outcomes at the time of recruitment.

Participants will be fitted as random effects. We will test for the presence of a carry-over effect (i.e. the possibility that results obtained during the second treatment phase are affected by what happened in the first treatment phase) by including a treatment-by-time period interaction in the model. We expect that this interaction effect will not be statistically significant.

Similar methods will be used for analyses of the additional objectives to ensure that (a) the data hierarchy is respected (i.e. repeated measurements within subjects) and (b) available data for all participants can be included. Regression models will be fitted to estimate differences in

secondary outcomes when treated with bezafibrate vs. placebo and, additionally, to quantify the extent to which more 'proximal' biomarkers are associated with more 'distal' clinical and symptomatic outcomes.

Non-adherence to random allocations will be documented and every effort will be made to include all randomised participants.

### 6.2 Subgroup analyses

No subgroup analysis is planned.

### 6.3 Frequency of analyses

The primary analysis will take place when follow-up is complete for all recruited participants. After phase 1 treatment has been completed by participants, a decision will be made by the Data Monitoring and Safety Committee as to whether an interim analysis should be conducted to assess the efficacy of phase I treatment. If so, confidential un-blinding will be performed by the study statistician to assess the response to bezafibrate. If an improved peak $VO_2$ (primary outcome) is demonstrated in ≥ 50% of participants receiving bezafibrate, phase 2 of the study will commence as planned. If no response is demonstrated, or a response is demonstrated in less than 50% of participants receiving bezafibrate, phase 2 of the study will not go ahead and the study will finish.

The DMSC will receive periodic reports on all adverse events to a timetable that the committee will set (together with the information about the medicinal product received if desired), together with any additional analyses the committee request.

### 6.4 Economic issues

No economic analyses are planned.

## 7. Trial management

The trial will be managed by the Clinical Trials and Evaluation Unit Bristol (CTEU Bristol), which is a UK Clinical Research Collaboration registered Clinical Trials Unit. The CTEU Bristol will prepare and submit all the relevant trial documentation for regulatory and governance approvals and prepare data collection forms, specify and deliver the randomisation scheme, develop and maintain the study data repository, check data quality as the trial progresses, monitor recruitment and carry out trial analyses in collaboration with the clinical investigators.

### 7.1 Day-to-day management

A Trial Management Group (TMG) will comprise: Chief Investigator, co-investigators and the trial manager. Members of the research team will be co-opted as required at different stages of the study. This group will meet at a frequency determined by the stage of the project and whether or not particular challenges in delivery need to be addressed. We expect that it will meet weekly during some periods where key decisions about the management of the study will have to be made.

The trial manager will be responsible for day-to-day management of the trial, which will be guided by the standard operating procedures and other infrastructure provided by the CTEU Bristol.

Potential trial participants will be identified through the Barth Syndrome Service run here in Bristol by a member of the research team. Informed consent from participants and their families will be sought by medically qualified doctors forming part of the research and/or care team, who have appropriate research training (GCP) and experience.

A research nurse will be responsible for collecting trial data. All members of the research team are responsible for ensuring the trial protocol is adhered to.

Trial Steering (TSC) and Data Monitoring and Safety Committees (DMSC) will be established to oversee the trial. We will identify appropriate nominees for these committees.

## 7.2 Monitoring of sites

### *7.2.1 Initiation visit*

Before the study commences training session(s) will be organised by CTEU Bristol for research team members and any other staff groups as appropriate. These sessions will ensure that personnel involved fully understand the protocol, CRFs and the practical procedures for the study.

### *7.2.2 Site monitoring*

As this is a single centre study there will be no site monitoring conducted by the CTEU Bristol.

## 7.3 Trial Steering Committee and Data Monitoring and Safety Committee

The TSC will have an independent Chair (an external Professor of Paediatrics with an interest in trial design), other clinical members with expertise in the disease area, and an adult with experience of Barth syndrome (lay member).

The DMSC will also be established to monitor recruitment of study participants, ethical issues of consent, quality of data, the incidence of adverse events, and any other factors that might compromise satisfactory completion of the study.

The DMSC will have an independent Chair. Membership will cover trial, statistics and relevant clinical expertise; all members will be completely independent of the trial research team. We will invite several international experts on Barth syndrome and national expert cardiac/haematology consultants to join. The DMSC will meet at least quarterly either face-to-face or by teleconference but the frequency may be increased if necessary. The Committee will make a recommendation to the TSC about continuation of the study after each meeting. The trial may be terminated early on the instruction of the DMSC

## 8. Safety reporting

Serious and other adverse events will be recorded and reported in accordance with the International Conference for Harmonisation of Good Clinical Practice (ICH GCP) guidelines and the Sponsor's Research Safety Reporting SOP (see Figure 2).

SAEs that require reporting to the Sponsor will be reported within 24 hours of the investigator becoming aware of the event. The sponsor will report SUSARs to regulatory authorities and copy all reports to CTEU Bristol.

Serious adverse events may prompt immediate withdrawal of the drug for a period at the discretion of the treating physician.

Note: Elective *treatment* taking place during the follow-up period that was planned prior to recruitment to the trial will not be reported as an unexpected SAE.

### 8.1 Expected adverse events

The Reference Safety Information (RSI) for the study is the SmPC for bezafibrate [14] (Summary of Product Characteristics Updated 10-May-2017; Generics UK T/A Mylan).

In Barth syndrome, adverse events are not unexpected and are not infrequent. The following list of events are 'anticipated' as part of the signs and symptoms of Barth syndrome and will not be reported to the Sponsor if the event is classified as serious, unless deemed related to bezafibrate. All other SAEs will be reported to Sponsor within 24 hours of the team becoming aware of the event.

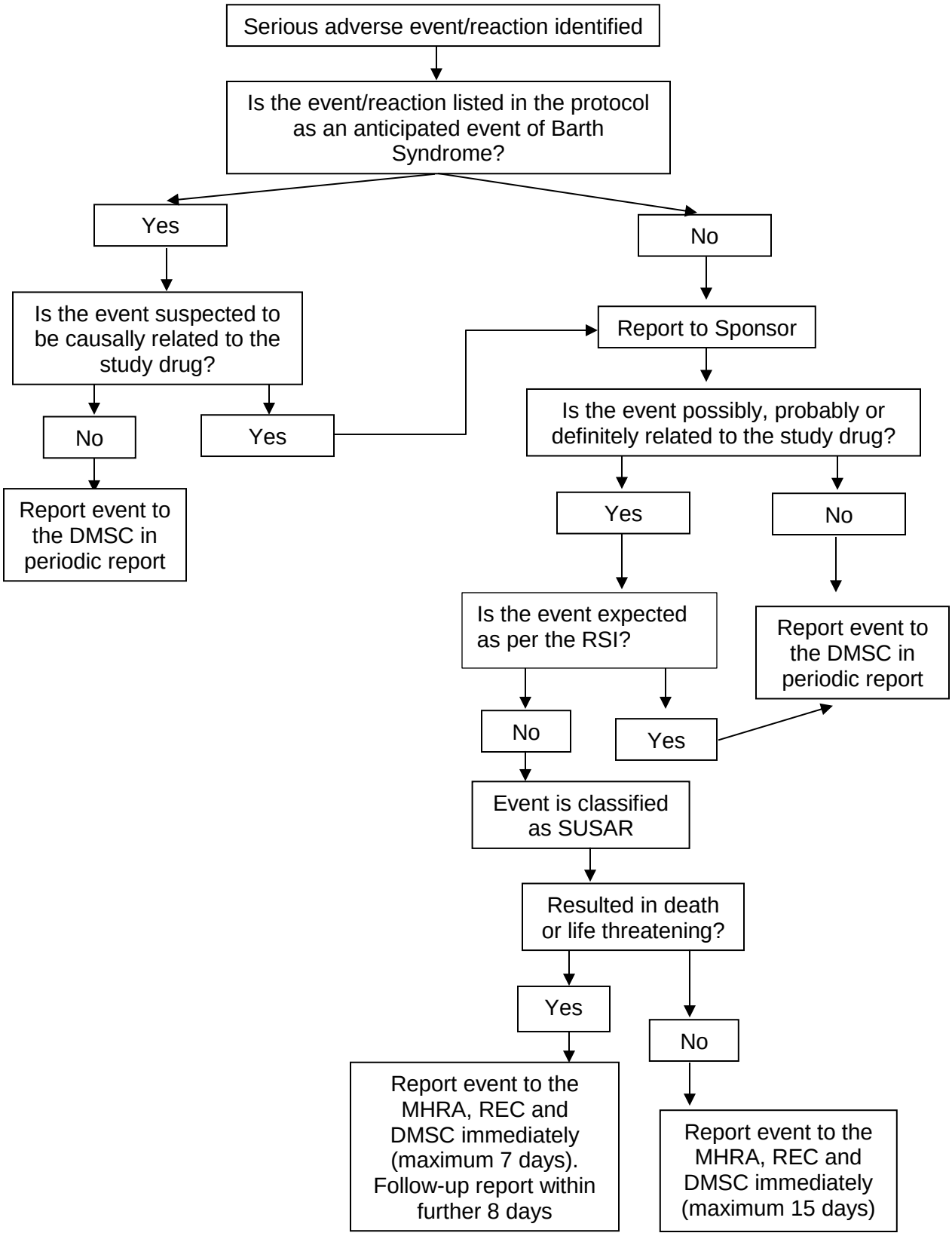
Data on all serious adverse events (including events in the list below) and their relatedness will be recorded in the patient's CRF and reported periodically to the DMSC for review.

- Small changes of dilated cardiomyopathy (DCM) (change in EF of <15%)
- Left ventricular non-compaction (LVNC)
- Prolonged corrected QT interval
- Proximal myopathy/weakness/fatigue
- Exercise intolerance
- Neutropaenia
- Aphthous ulcers and sore gums
- Bacterial skin infections
- Hypocholesterolaemia
- Hypoglycaemia (primarily in infants)
- Episodic or chronic diarrhoea

Adverse drug reactions (ADRs) as listed in section 4.8 the SmPC for bezafibrate [14] (Summary of Product Characteristics Updated 10-May-2017; Generics UK T/A Mylan) are 'expected' events as a result of taking the study medication and will be used to make 'expectedness' assessments of SAEs.

Adverse event data will be collected from the time of consent until the end of the 1 month follow-up period.

**Figure 2 Serious adverse event reporting flow chart**



### 8.2 Period for recording serious adverse events

Data on serious adverse events will be collected from consent until the end of the follow-up period.

## 9. Ethical considerations

### 9.1 Review by an NHS Research Ethics Committee

Ethics review of the protocol for the trial and other trial related essential documents (e.g. PIL and consent form) will be carried out by a UK NHS Research Ethics Committee (REC).

Any amendments to these documents, after a favourable opinion from the REC has been given, will be submitted to the REC for approval prior to implementation.

### 9.2 Risks and anticipated benefits

**Potential benefits to participants:** There is no current treatment for Barth syndrome itself and manifested symptoms are treated individually on a clinical basis. Therefore the use of bezafibrate in our clinical trial is the first potential treatment available in the UK (a trial of a subcutaneously injectable drug aiming to stabilise cardiolipin commenced in the USA in July 2017) for those affected by Barth syndrome. Barth syndrome patients, parents and guardians who attend the NHS Barth Syndrome Service clinics in Bristol are already aware of the potential benefits of bezafibrate, but the beneficial effects of bezafibrate have not yet been proven in this population and care will be taken to ensure that potential participants are aware of the equipoise of the study.

**Potential harms to participants:** Bezafibrate has a number of side-effects that may be experienced by participants taking the drug, as detailed below. In addition to this, participants are required to make additional visits to Bristol as part of the study, which will cause some inconvenience and participants will also be required to undergo additional tests that they would not usually be asked to do.

The exercise and MRI/MRS testing to be used is modelled on the approach of Professor Todd Cade and team (University of Washington, St. Louis, USA) who have previously conducted extensive exercise testing in both children and adults with Barth syndrome [18]. Measurement of muscle bioenergetics required use of a proprietary MRI coil designed and synthesised by that team and whose manufacture is being reproduced in Bristol by Dr Paul Warr at the University of Bristol's Department of Electrical & Electronic Engineering, under the supervision of Professor Risto Kauppinen, University of Bristol, an expert in MR imaging. Advice is also being provided by CRIC medical physicists and the manufacturer regarding compatibility and configuration with the scanner. The coil will have undergone extensive safety testing before being presented to the CRIC Board for approval. The UH Bristol Medical Equipment Management Organisation will also independently verify the suitability of the coil as part of this process.

Professor Cade's studies included 20 children/adolescents with Barth Syndrome and 23 controls (detailed in reference [18]). No untoward events have occurred. Therefore we do not have reason to believe that exercise or MRI/MRS testing will result in potential harm to participants, but a paediatric cardiologist will be present during the exercise testing and during

echocardiography and other appropriately medically qualified staff will attend the exercise testing during MRI/MRS scanning. Additional safety monitoring during bicycle exercise will be provided by 12-lead ECG (“stress ECG".) This non-invasive method is routinely used in paediatric cardiology clinical practice.

Participants able to tolerate the MRS assessments will be required to spend approximately an hour in the scanner, which may be uncomfortable.

Please refer to the Bezafibrate 200 mg Film-coated Tablets Summary of Product Characteristics updated 10-May-2017 (Generics UK T/A Mylan) for possible adverse effects of bezafibrate

No adverse effects are expected from the placebo intervention

*Benefits to society:* This trial can be expected to help educate those affected by the disease and their families about the importance of effective trial design, treatment adherence and regular assessment in establishing future therapies to benefit both their personal health and that of others with the same disease. The results of the study will also advance knowledge of the treatment of Barth syndrome patients and help to inform future studies.

### 9.3 Informing potential study participants of possible benefits and known risks

Information about possible benefits and risks of participation will be described in the PIL.

### 9.4 Obtaining informed consent from participants

Participants aged 16 years and older will be required to complete a written informed consent form before being enrolled in the study. For participants ***under*** the age of 16 years (≤15 years), written informed consent will be obtained from the child’s parents/legal guardian but information will be given to the child about the study and its risks and benefits, according to their level of understanding and by staff with experience of recruiting children. The wishes of the child will be taken into consideration by the investigator in deciding whether or not they should be included in the trial. Children with sufficient understanding and capacity (usually aged 11-15) may be asked to formally document their ‘assent’ in written format if this is deemed appropriate.

The Chief Investigator and/or a research nurse will be responsible for the overall informed consent process, which will be described in detail in the Trial Manual. See section 7.1 for details on who will take written informed consent.

### 9.5 Co-enrolment

Potentially eligible participants will be excluded from the study if they are already participating in another interventional research study, due to effects of the other intervention that may be observed on our study outcomes and also for participant safety. In addition, the small number of patients we will recruit will not enable equal distribution of participants involved in other interventional research across the groups.

Participants already enrolled on this study should not be enrolled on any other interventional research study until after they have completed the follow-up period.

Participants can be enrolled in observational studies during their time in CARDIOMAN.

## 10. Research governance

This study will be conducted in accordance with:

- The Medicine for Human Use (Clinical Trial) Regulations 2004
- International Conference for Harmonisation of Good Clinical Practice (ICH GCP) guidelines
- UK Policy Framework for Health and Social Care Research
- European Union Directive 2001/20/EC on clinical trials

### 10.1 Sponsor approval

Any amendments to the trial documents must be approved by the sponsor prior to submission to the REC and MHRA.

### 10.2 NHS approval

Agreement from the local NHS Trust is required prior to the start of the trial.

Any amendments to the trial documents approved by the HRA/REC/MHRA will be submitted to the Trust for information or approval as required.

### 10.3 Investigators' responsibilities

Investigators will be required to ensure that local research approvals have been obtained and that any contractual agreements required have been signed off by all parties before recruiting any participant. Investigators will be required to ensure compliance to the protocol and study manual and with completion of the CRFs. Investigators will be required to allow access to study documentation or source data on request for monitoring visits and audits performed by the sponsor or CTEU Bristol or any regulatory authorities.

Investigators will be required to read, acknowledge and inform their trial team of any amendments to the trial documents approved by the HRA/REC/MHRA that they receive and ensure that the changes are complied with.

### 10.4 Monitoring by sponsor

The study will be monitored and audited in accordance with the sponsor's Monitoring and Oversight of Research Activity SOP, which is consistent with the UK Policy Framework for Health and Social Care Research and the Medicines for Human Use (Clinical Trials) Regulations 2004. All study related documents will be made available on request for monitoring and audit by the sponsor, the relevant REC and for inspection by the MHRA or other licensing bodies.

### 10.5 Indemnity

This is an NHS-sponsored research study. For NHS sponsored research if there is negligent harm during the clinical trial when the NHS body owes a duty of care to the person harmed,

NHS Indemnity covers NHS staff, medical academic staff with honorary contracts, and those conducting the trial. NHS Indemnity does not offer no-fault compensation and is unable to agree in advance to pay compensation for non-negligent harm. Ex-gratia payments may be considered in the case of a claim.

### 10.6 Clinical Trial Authorisation

Bezafibrate is classed as an investigational medicinal product (IMP) and a Clinical Trial Authorisation from the MHRA must be in place before starting the trial.

## 11. Data protection and participant confidentiality

### 11.1 Data protection

Data will be collected and retained in accordance with the UK Data Protection Act 2018 and The General Data Protection Regulation 2016.

### 11.2 Data handling, storage and sharing

#### *11.2.1 Data handling*

Data will be collected on paper Case Report Forms (CRFs) and entered onto a purpose designed Excel spreadsheet with data validation and cleaning carried out on an ongoing basis throughout the trial.

Access to the study spreadsheet(s) will be password-protected and only accessible to members of the research team. The spreadsheet will be held on NHS networked servers, with participants being identified using their name and unique study identifier. Study data transferred electronically to the University of Bristol network for statistical analyses will be pseudonymised and transferred via a secure network.

#### *11.2.2 Data storage*

All study documentation will be retained in a secure location during the conduct of the study and for 25 years after the end of the study when all patient identifiable paper records will be destroyed by confidential means. Where trial related information is documented in the medical records, these records will be identified by a label bearing the name and duration of the trial in accordance with UHBristol policy. In compliance with the MRC Policy on Data Preservation, relevant 'meta'-data about the trial and the full dataset, but without any participant identifiers other than the unique participant identifier, will be held indefinitely (University server). A secure electronic 'key' with a unique participant identifier, and key personal identifiers (e.g. name, date of birth and NHS number) will also be held indefinitely, but in a separate file and in a physically different location (NHS hospital server). These will be retained because of the potential for the raw data to be used subsequently for secondary research.

#### *11.2.3 Data sharing*

Data will not be made available for sharing until after publication of the main results of the study. Thereafter, if appropriate consent has been given by the participant, a file containing individual patient data with identifiers removed will be made available for secondary research, conditional

on assurance from the secondary researcher that the proposed use of the data is compliant with the MRC Policy on Data Preservation and Sharing regarding scientific quality, ethical requirements and value for money. A minimum requirement with respect to scientific quality will be a publicly available pre-specified protocol describing the purpose, methods and analysis of the secondary research, e.g. a protocol for a Cochrane systematic review. A second file containing patient identifiers would be made available for record linkage or a similar purpose, subject to confirmation that the secondary research protocol has been approved by a UK REC or other similar, approved ethics review body.

## 12. Dissemination of findings

We will report our intention to conduct this study and major aspects of the trial design through the websites of the Barth Syndrome Trust and Barth Syndrome Service and the Journal of the Barth Syndrome Foundation (BSF, USA). Progress reports on design, recruitment and results will be presented to the biennial BSF Medical, Scientific and Family Conferences.

When all of the data has been collected and analysed we will hold a social gathering after one of the Barth Syndrome Service clinics to feed the information back to the patient/families directly and to thank them for their participation. We will send a lay summary to all people/families in the UK with Barth Syndrome.

The Barth Syndrome Service uses a certified private provider patient information site (Patients Know Best) to allow patients and medical carers easy online access to their personal medical information and treatment plans. A lay summary of the results will be posted to each patient's personal pages, for both trial participants and non-participants, as a permanently available, easily accessible record.

Decisions on the most appropriate leads for specific dissemination/ exploitation activities will be made by the Steering Group. Information will be made freely available through web sites. Suitable summaries of the research findings will be licenced to individual web sites to ensure that the material remains available for a fixed period of time regardless of hit rates.

Findings from the study will be submitted for publication in a peer-reviewed journal, selecting one with open access arrangements, under the NIHR Terms of Agreement, in order to allow rapid dissemination to the Barth syndrome community. We would also present the findings at national/international paediatric, paediatric cardiology and metabolic disease conferences.

The conduct and findings of this trial will be discussed by videoconference/meetings of the Scientific and Medical Advisory Board of the Barth Syndrome Foundation, of which Dr Toth and Professor Steward are members. If it were decided that further trials were necessary, for example if important endpoints showed promising trends but not statistical significance or detailed dose finding studies were deemed necessary, we would discuss the potential for international collaboration at those meetings. We are cognisant that it would have been ideal to conduct this trial in the first instance as an international collaboration (e.g. for sample size, statistical power) but this has not been possible due to the unavailability of bezafibrate in the USA and other considerations. We are hopeful that such barriers will be removed in the future to allow more effective European/US cooperation on this and other very rare diseases.

## 13. References


1. Ren, M., *A mouse TAZ-knockdown model of Barth syndrome cardiomyopathy: pathophysiology and potential treatments*, in Barth Syndrome Foundation Medical, Scientific and Family Conference. 2012.
2. Clarke, S.L., et al., *Barth syndrome.* Orphanet J Rare Dis, 2013. 8: p. 23.
3. Spencer, C.T., et al., *Ventricular arrhythmia in the X-linked cardiomyopathy Barth syndrome.* Pediatr Cardiol, 2005. 26(5): p. 632-7.
4. Huang, Y., et al., *The PPAR pan-agonist bezafibrate ameliorates cardiomyopathy in a mouse model of Barth syndrome.* Orphanet J Rare Dis, 2017. 12(1): p. 49.
5. Schafer, C., et al., *The Effects of PPAR Stimulation on Cardiac Metabolic Pathways in Barth Syndrome Mice.* Front Pharmacol, 2018. 9: p. 318.
6. Goldenberg I, B.V., Tennenbaum A, Tanne D, Behar S, Guetta V, *Long-term benefit of highdensity lipoprotein cholesterol-raising therapy with bezafibrate: 16-year mortality follow-up of the bezafibrate infarction prevention trial.* Arch Intern Med, 2009. 169(5): p. 508-14.
7. Wheeler, K.A., et al., *Double blind trial of bezafibrate in familial hypercholesterolaemia.* Arch Dis Child, 1985. 60(1): p. 34-7.
8. Nagasaka, H., et al., *Effects of bezafibrate on dyslipidemia with cholestasis in children with familial intrahepatic cholestasis-1 deficiency manifesting progressive familial intrahepatic cholestasis.* Metabolism, 2009. 58(1): p. 48-54.
9. Becker, M., D. Staab, and K. Von Bergman, *Long-term treatment of severe familial hypercholesterolemia in children: effect of sitosterol and bezafibrate.* Pediatrics, 1992. 89(1): p. 138-42.
10. Engelen, M., et al., *Bezafibrate for X-linked adrenoleukodystrophy.* PLoS One, 2012. 7(7): p. e41013.
11. Bonnefont, J.P., et al., *Long-term follow-up of bezafibrate treatment in patients with the myopathic form of carnitine palmitoyltransferase 2 deficiency.* Clin Pharmacol Ther, 2010. 88(1): p. 101-8.
12. Patel, K.R., et al., *Clinical trials of resveratrol.* Ann N Y Acad Sci, 2011. 1215: p. 161-9.
13. Timmers, S., et al., *Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans.* Cell Metab, 2011. 14(5): p. 612-22.
14. *Summary of Product Characteristics Updated 10-May-2017* Generics UK T/A Mylan, Bezafibrate 200 mg Film-coated Tablets Available from: https://www.medicines.org.uk/emc/product/2544/smpc
15. Ritchie J, *Applied data analysis for applied policy research*. In Analyzing qualitative data, ed. S. L. 1994, London: Routledge.
16. Machin D., *Sample size tables for clinical studies*. 2nd edition, ed. F.P. Campbell M, Pinol A. 1997, Oxford: Blackwell.
17. Graham, D.J., et al., *Incidence of hospitalized rhabdomyolysis in patients treated with lipid-lowering drugs.* JAMA, 2004. 292(21): p. 2585-90.
18. Bashir, A., et al., *Impaired cardiac and skeletal muscle bioenergetics in children, adolescents, and young adults with Barth syndrome.* Physiol Rep, 2017. 5(3).

## Amendments to protocol

| Amendment number | Version number of new protocol | Date of new version of protocol | Brief summary of change | Date of ethical approval (or NA if non-substantial) | Date of MHRA approval (or NA if non-substantial) |
|---|---|---|---|---|---|
| 1.0 | 3.0 | 04/10/2018 | • Background information updated<br>• Change of washout period from one month to a minimum of 1 month<br>• Clarification that the decision for an interim analysis will lie with the DMSC<br>• Additional exclusion criteria updated<br>• Intervention section updated with new drug details<br>• Clarification on safety reporting with the addition of the bezafibrate SmPC as the RSI<br>• Addition of information regarding MRS testing<br>• Additional statement on GDPR Regulations | | |

**Appendix 1**

**Echocardiography Protocol:**

1.) Participants and parents welcomed to exercise laboratory by research staff and familiarised with facilities.
2.) Functional trans-thoracic echocardiography will be performed at rest in a semi-recumbent position
3.) Start of exercise practise and familiarisation on exercise bike with appropriate rest thereafter
4.) 10 ECG stickers will be attached to the patient's chest and connected to an ECG monitor that is linked to the metabolic exercise software platform during exercise and recovery
5.) Start of exercise test and acquisition of echocardiographic, oxygen consumption and NIRS data. A full exercise protocol will take approx. 21 mins to complete. Oxygen consumption and NIRS data will be recorded continuously, echocardiographic data will be acquired during exercise at every exercise stage, that is every 3 minutes, at maximal 7 exercise stages. Each acquisition will take approximately one minute. For detailed exercise protocol please see below. Heart rate, blood pressure, oxygen saturations and NIRS will be acquired at each stage by standard non-invasive recording.
6.) Cessation of exercise if protocol completed, participant wishes to stop or research staff determine the test deleterious.
7.) Continuous oxygen consumption and NIRS data acquisition until 10 min recovery. Echocardiographic data acquisition at rest at 2 min and 10 min recovery
8.) Participants can recover and have a drink/ food as requested
9.) End of visit

Participant's baseline heart rate (HR) and blood pressure (BP) are recorded at rest and at peak exercise (Dinamap Pro 100V2, GE Medical Systems Information Technologies 2002, Tampa, Florida, USA).

Near infrared spectroscopy (NIRS) is used to measure muscle deoxygenation (HHb) during exercise (Artinis PortaMon near-infrared spectrometer, Artinis Medical Systems, The Netherlands). The device is secured over the vastus lateralis muscle, using the midpoint of the anterior superior iliac spine and the head of the fibula as a reference point.

Exercise stress test is performed on an electronically braked GE Healthcare exercise echocardiography eBike EL (GE Medical Systems Information Technologies GmbH, Freiburg, Germany) whilst echocardiography is simultaneously performed by a Cardiologist. A step protocol is used, with each child beginning on a baseline of 0 watts, and the intensity increased by 25 watts every 3 minutes (modified McMaster protocol). Exercise stages of three minutes are used to obtain detailed "steady state" information for each exercise stage and facilitate echocardiographic image acquisition. Children are encouraged to cycle at 60 revolutions per minute (rpm) throughout. Children are told to cycle until completion/ the point of voluntary exhaustion. The exercise test is terminated when the child is unable to continue due to exhaustion or when they cannot sustain a cadence of 60 ± 5 rpm.

Oxygen consumption is measured using a gas cart (Metalyzer 3B Cortex, Biophysik, Leipzig, Germany). The device was calibrated using a known reference gas before testing and a 3 litre calibration syringe was used to calibrate the turbine volume transducer (Hans Rudolph, Kansas City, MO). The gas cart provided breath-by-breath gas exchange analysis, averaged to 10 second time intervals. The highest 10 second average VO2 represented that individuals VO2peak. Average data for each stage is expressed as the average of the last 30 seconds of each workload. The Borg Scale is used to measure the rating of perceived exertion (RPE) at the end of each 3 minute interval, with the children asked to subjectively indicate which grading (scale out of 10) is relevant to them at that point in the test.

Functional trans-thoracic echocardiography at rest is performed by a Cardiologist following the guideline of the American Society of Echocardiography (1) and using the following hardware: Vivid Q or E9, General Electric, cardiac transducers 4-6 Mhz, GE Healthcare, Little Chalfont, UK). Left Ventricular (LV) systolic function during rest and exercise is assessed using short axis and long axis fractional shortening (FS), Pulse Wave –Tissue Doppler Imaging (PW-TDI) derived myocardial systolic velocities and 2-D myocardial strain analysis (speckle tracking). For longitudinal and radial strain analysis images of the left ventricle are obtained in apical 4 chamber and parasternal short axis views. Images for strain analysis are obtained at a rate of 40-90 frames per minute during rest and exercise. Diastolic LV function is assessed using Pulse Wave –Tissue Doppler Imaging (PW-TDI) derived myocardial diastolic velocities and PW Doppler mitral valve inflow velocities. Fast image acquisition during exercise is facilitated using the GE exercise stress protocol GE software. Functional echocardiographic quantification of left ventricular function is processed offline using the Q analysis research tool of ECHO PAC offline software (EchoPac, GE Healthcare, Little Chalfont, UK).

Echocardiography protocol:
Rest:
Exercise and recovery: 7 images per exercise stage:

1. 2-D 4 Chamber view LV
2. Tissue Doppler Imaging (TDI) 4 Chamber view
3. Pulse Wave (PW) TDI lateral LV 4 chamber view
4. Mitral valve inflow PW Doppler trace
5. Aortic outflow PW Doppler trace
6. 2-D parasternal short-axis view (base)
7. 2-D parasternal short axis view (apex)

1. Lopez L, Colan SD, Frommelt PC, Ensing GJ, Kendall K, Younoszai AK, *et al*. Recommendations for quantification methods during the performance of a pediatric echocardiogram: a report from the Pediatric Measurements Writing Group of the American Society of Echocardiography Pediatric and Congenital Heart Disease Council. J Am Soc Echocardiogr. 2010 May;23(5):465-95; quiz 576-7.

1. M, R., *A mouse TAZ-knockdown model of Barth syndrome cardiomyopathy: pathophysiology and potential treatments*, in *Barth Syndrome Foundation Medical, Scientific and Family Conference*. 2012.
2. Clarke, S.L., et al., *Barth syndrome.* Orphanet J Rare Dis, 2013. **8**: p. 23.
3. Spencer, C.T., et al., *Ventricular arrhythmia in the X-linked cardiomyopathy Barth syndrome.* Pediatr Cardiol, 2005. **26**(5): p. 632-7.

4. Huang, Y., et al., *The PPAR pan-agonist bezafibrate ameliorates cardiomyopathy in a mouse model of Barth syndrome.* Orphanet J Rare Dis, 2017. **12**(1): p. 49.
5. Schafer, C., et al., *The Effects of PPAR Stimulation on Cardiac Metabolic Pathways in Barth Syndrome Mice.* Front Pharmacol, 2018. **9**: p. 318.
6. Goldenberg I, B.V., Tennenbaum A, Tanne D, Behar S, Guetta V, *Long-term benefit of highdensity lipoprotein cholesterol-raising therapy with bezafibrate: 16-year mortality follow-up of the bezafibrate infarction prevention trial.* Arch Intern Med, 2009. **169(5)**: p. 508-14.
7. Wheeler, K.A., et al., *Double blind trial of bezafibrate in familial hypercholesterolaemia.* Arch Dis Child, 1985. **60**(1): p. 34-7.
8. Nagasaka, H., et al., *Effects of bezafibrate on dyslipidemia with cholestasis in children with familial intrahepatic cholestasis-1 deficiency manifesting progressive familial intrahepatic cholestasis.* Metabolism, 2009. **58**(1): p. 48-54.
9. Becker, M., D. Staab, and K. Von Bergman, *Long-term treatment of severe familial hypercholesterolemia in children: effect of sitosterol and bezafibrate.* Pediatrics, 1992. **89**(1): p. 138-42.
10. Engelen, M., et al., *Bezafibrate for X-linked adrenoleukodystrophy.* PLoS One, 2012. **7**(7): p. e41013.
11. Bonnefont, J.P., et al., *Long-term follow-up of bezafibrate treatment in patients with the myopathic form of carnitine palmitoyltransferase 2 deficiency.* Clin Pharmacol Ther, 2010. **88**(1): p. 101-8.
12. Patel, K.R., et al., *Clinical trials of resveratrol.* Ann N Y Acad Sci, 2011. **1215**: p. 161-9.
13. Timmers, S., et al., *Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans.* Cell Metab, 2011. **14**(5): p. 612-22.
14. Mylan, G.U.T.A. *Summary of Product Characteristics Updated 10-May-2017* Available from: https://www.medicines.org.uk/emc/product/2544/smpc
15. J, R., *Applied data analysis for applied policy research*. In Analyzing qualitative data, ed. S. L. 1994, London: Routledge.
16. D, M., *Sample size tables for clinical studies*. 2nd edition ed, ed. F.P. Campbell M, Pinol A. 1997, Oxford: Blackwell.
17. Graham, D.J., et al., *Incidence of hospitalized rhabdomyolysis in patients treated with lipid-lowering drugs.* JAMA, 2004. **292**(21): p. 2585-90.
18. Bashir, A., et al., *Impaired cardiac and skeletal muscle bioenergetics in children, adolescents, and young adults with Barth syndrome.* Physiol Rep, 2017. **5**(3).