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

CARDIOMAN

Statistical Analysis Plan

Role	Name	Signature	Date
Study Statistician			
Authorising Statistician			
Chief Investigator			

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1. Introduction

1.1 Summary of document

1.1.1 Scope

The statistical analysis plan for the CARDIOMAN study has been written in accordance with BTC standard operating procedures, the CONSORT statement, and International Conference on Harmonisation (ICH) Statistical Principles for Clinical Trials E9, by Laura Collett, Medical Statistician / Senior Research Associate at BTC, under the supervision of Professor Chris Rogers, Director of BTC, and covers all final statistical analyses to be performed, outlined in the study protocol found study master file.

1.1.2 Planned analyses and dissemination

An end of study report will be produced once phase 2 of the study has finished, once the second 4 month treatment phase has finished and outcome data collected, and will be disseminated to the TMG when all pre-specified final analyses have been performed. An independent joint data monitoring and safety committee and trial steering committee (DMSC/TSC) will review the safety and ethics of the study. Short blinded study update reports including safety and compliance will be produced and disseminated at regular intervals during the study. The DMSC/TSC have the authority to recommend that the study stops if deemed necessary based on the observed data.

1.2 Background of study

1.2.1 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 statistician 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 Bristol University and Great Ormond St Hospital to see the effect of bezafibrate on participant's 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 participant's 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.

1.2.2 Aims and objectives

It is hypothesised that bezafibrate will modify the cellular ratio of monolysocardiolipin to L4-cardiolipin, ameliorating disease phenotype in those living with the disease, providing the drug is free of significant side-effects at clinically effective doses.

It is also hypothesised that bezafibrate (and/or resveratrol in-vitro) may have beneficial effects independent of any effect on cardiolipin ratio due to their abilities to influence a range of mitochondrial pathologies.

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.

2. Study methods

2.1 Design

This study is a double-blinded, randomised, placebo-controlled crossover study. Treatment will be given in two 4-month long phases and a 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 (bezafibrate or placebo).

2.2 Randomisation

A randomisation list will be created, numbered by study ID, with a 1:1 ratio of the allocations: bezafibrate followed by placebo, or placebo followed by bezafibrate. The allocation will be according to permuted block randomisation, with blocks of varying size. This list of study IDs and corresponding medication order will be provided to the clinical trials pharmacy department.

N.B. This method of randomisation does not comply with the requirement of an internet-based randomisation system supplied by an MHRA approved provider (e.g. Sealed Envelope) as described in the Bristol CTEU SOP ST 005 'Development of a Randomisation System', but an internet-based system was thought to be prohibitively expensive for so few patients.

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 given the participant's study ID and phase of 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.

2.3 Framework

The difference between the treatments will be compared according to a superiority framework.

2.4 Sample size

This is a single centre study to be performed in patients attending the NHS National Barth Syndrome Service. 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: square root of 2 times the within subject SD. In a crossover trial, all participants act as 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 type I error of 5%. 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.

2.5 Blinding

All participants, their clinical care team, their research nurse(s) response for follow-up, and all BTC staff will be unaware of the allocation order.

3. Populations

3.1 Study populations

3.1.1 Inclusion criteria

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

3.1.2 Exclusion criteria

- Known hypersensitivity to bezafibrate, to any component of the product or to other fibrates
- Known photoallergic of phototoxic reactions to fibrates
- Hepatic dysfunction and/or liver function tests greater than 2x normal
- A shortening fraction of <25 (or a significant drop in shortening fraction in the previous year)
- Documented atrial or ventricular arrhythmia (atrial/ventricular tachycardia or atrial/ventricular fibrillation) that has not been stabilised with treatment
- Renal impairment (creatinine clearance < 90 mL/min)
- Pre-existing known gallbladder disease
- Recent unspecified significant deterioration in general health
- Prisoners and adults lacking capacity to provide informed consent

3.2 Data sources

All study data will be collected on case report forms (CRFs), with the exception of the mitochondrial data required to answer the MLCL/L4-CL ratio / cardiolipin profile outcomes, the MRS data required to answer PCr/ATP ratio and skeletal muscle oxidative function outcomes, the MRI data required to answer the MRI cardiac function outcomes, and the mitochondrial data. These data will be obtained and recorded in spreadsheets by the laboratory staff, sent to the study statistician, and linked using studyid.

3.3 Analysis populations

3.3.1 Intention-to-treat population

All summaries and analyses of the primary and secondary outcomes will be conducted on the intention-to-treat (ITT) population. The ITT population will consist of all participants and periods, included according to the sequence they were randomised to and the treatment they were randomised in that given period, regardless of whether they are ineligible, prematurely discontinue treatment or are otherwise protocol violators/deviators.

3.3.2 Per-protocol population

In addition to the ITT analysis, a complier average casual effect (CACE) analysis will be considered for the primary outcome if there are a considerable number of major protocol deviators (>20% of participants). The number of protocol deviators and the extent of the protocol deviations will be monitored.

Major protocol deviators include the following:

- Incorrect unblinding procedure
- Any serious breaches to the protocol, defined as those events that affect the confidentiality or safety of participants, or the integrity of the trial
- Participant does not meet the study eligibility criteria, but receives a single dose of study treatment

In order to assess the utility of a per-protocol analysis, the proportion of protocol deivators will be assessed, without reference to outcome data, through a blinded examination of the population at the time of analysis by the study statistician in co-operation with other members of the TMG.

3.3.3 Safety population

The safety population will consist of all randomised participants, included according to the treatment they actually received in each treatment period, and who have received at least one dose of trial treatment.

3.4 Withdrawals

Participants can withdraw/be withdrawn from treatment and/or follow-up at any stage of the trial at their own discretion or that of the treating clinician. All participants withdrawn from the trial, in addition to those found to be ineligible post-randomisation, will be continued to followed up until the end of the study, unless they withdraw full consent for further follow-up data to be collected. All data previously collected by the participant will be used in the analysis, unless they have withdrawn full consent from collected data to be used.

4. Statistical analyses and report content

4.1 General considerations

Statistical analysis is the responsibility of the BTC study statistician and all analyses will be carried out using the most recent version of the statistical software at the time of analysis.

Where treatments are being compared formally using statistical modelling, the placebo group will act as the reference category, in order for interpretations to be in the form: the effect of bezafibrate in comparison with placebo. All applicable statistical tests will be 2-sided and will be performed using a 5% significance level, with the exception of tests for interactions that will be performed using a 10% significance level, and confidence interval will be 95% unless otherwise stated.

No formal adjustment will be made for multiple testing, but consideration will be taken in interpretation of results to reflect the number of statistical tests performed and the consistency, magnitude and direction of treatment estimates for different outcomes.

4.2 General calculations

Unless otherwise stated, all percentages will be calculated using the total number of participants with data available for that variable, with any missing data mentioned in footnotes. For categorical and binary data, all percentages will be rounded to at most 1 decimal place, and for continuous measures, these will be summarised to one more decimal place than the data is collected. P-values >0.001 will be summarised to 2 significant figures, and those <0.001 will be reported as <0.001).

4.3 Outcomes

4.3.1 Primary outcome

- Peak VO_2

4.3.2 Secondary outcomes

Secondary outcome measures include:

- MLCL/L4-CL ratio / cardiolipin profile
- PCr/ATP ratio
- Skeletal muscle oxidative function
- Quality of life
- Absolute neutrophil count
- Amino acid expression
- Cardiac function
- Lymphocyte mitochrondria
- Arrhythmia profile

4.4 Definition and derivation of the outcomes

4.4.1 Primary outcome

Peak VO_2 is defined as the peak oxygen consumption on bicycle ergonometry at baseline and in the final week of each treatment period.

4.4.2 Secondary outcomes

MLCL/L4-CL ratio / cardiolipin profile

Participants will be assessed for their monolysocardiolipin/tetralinoleoyl-cardiolipin (MLCL/L4-CL) ratio calculated using results from mitochondrial assessments carried out at baseline and in the final week of each treatment period.

PCr/ATP ratio

Participants will be assessed for their phosphocreatine/adenosine triphosphate (PCr/ATP) ratio, assessed via myocardium and oxidative function using a ^{31}P magnetic resonance spectroscopy (MRS). These assessments will only be carried out in the final week of the second treatment period due to delays in approving the technology.

Skeletal muscle oxidative function

Participants will be assessed for their skeletal muscle oxidative function using a ^{31}P MRS in the final week of the second treatment period.

Quality of life

Quality of life (QoL) will be assessed using age-appropriate PedsQL questionnaires at baseline and in the final week of each treatment period. The PedsQL will be scored according to guidelines set out in the PedsQL user [1].

Absolute neutrophil count

Neutrophil count will gained from blood tests at baseline and as part of the monthly blood tests taken throughout both treatment periods.

Amino acid expression

Amino acid expression will be derived from participants' arginine and cysteine levels, and will be assessed at baseline and as part of the monthly blood tests throughout both treatment periods.

Cardiac function

Participants' cardiac function will be assessed using echocardiogram, at rest and during exercise, at baseline and in the final week of each treatment period.

Lymphocyte mitochondria

Participants' lymphocyte mitochondria will be assessed by electron microscopy, at baseline and in the final week of each treatment period.

Arrhythmia profile

Participants' arrhythmia profile will be assessed using 12-lead electrocardiogram (ECG), at rest and during exercise, at baseline and in the final week of each treatment period.

4.5 Analysis of the outcomes

4.5.1 Primary outcome

Peak VO_2

Peak VO_2 (VO_2 max (ml/kg/min)) will be the primary analysis, and will be analysed formally using a mixed linear regression model to compare bezafibrate against placebo, adjusting for period as a fixed effect (exploring interactions where necessary) and participants as random effects. Nested models will be compared using the likelihood ratio test. Model assumptions will be tested using standard methods, including residual plots etc., and a test for carry-over will be carried out by including treatment sequence in the model. If these assumptions do not appear to be valid then alternative methods will be explored.

Rest VO_2 will also be analysed formally in a similar way to peak VO_2. Peak wattage achieved (when peak VO_2 is measured) will be assessed for consistency within participants across time periods by calculating the correlation.

Peak and rest VO_2, and 2 and 6 min recovery VO_2 will also be summarised descriptively using means and standard deviations (SDs) (or medians and interquartile ranges (IQRs) depending on the distribution) at each period separately, and graphical representations of the distribution will be presented by treatment and period.

4.5.2 Secondary outcomes

MLCL/L4-CL ratio / cardiolipin profile

MLCL/L4-CL ratio will be analysed formally using a mixed linear regression model to compare bezafibrate against placebo, in the same way, using the same methods as the primary analysis of peak VO_2 above.

MLCL/L4-CL ratios will also be summarised descriptively using means and SDs (or medians and IQRs) by treatment and period, and graphical representations of the distributions will be presented by treatment and period.

Cardiolipin profile will be analysed visually by the laboratory team and will not form part of the statistical analysis.

PCr/ATP ratio

PCr/ATP ratio for the second period will be summarised descriptively using a mean and SD (or median and IQR) by treatment and overall, and graphical representations of the distribution will be presented by treatment and overall.

Skeletal muscle oxidative function

Skeletal muscle oxidative function (a panel of quantities to determine function) for the second period will be summarised descriptively using means and SDs (or medians and IQRs) by treatment and overall, and graphical representations of the distribution will be presented by treatment and overall.

Quality of life

Quality of life (QoL) index score, derived from value sets calculated via the responses for each domain of the PedsQL questionnaire, will be analysed formally using a mixed linear regression model to compare bezafibrate against placebo, in the same way, using the same methods as the primary analysis of peak VO_2 above, for participants of each age group and parents/carers separately.

Participants' and parents'/carers' QoL scores for each domain of the questionnaire will also be summarised descriptively using means and SDs (or medians and IQRs) or counts and percentages (where appropriate) by treatment and period, and graphical representations of the distributions will be presented by treatment and period.

Absolute neutrophil count

Absolute neutrophil count will also be analysed formally using a mixed linear regression model to compare bezafibrate against placebo, in the same way, using the same methods as the primary analysis of peak VO_2 ratio above.

Amino acid expression

Plasma arginine and plasma cysteine values will also be analysed formally using mixed linear regression models to compare bezafibrate against placebo, in the same way, using the same methods as the primary analysis of peak VO_2 ratio above.

Cardiac function

Cardiac function is measured by ECHO and MRI. The main ECHO parameters of interest reported at rest, exercise and 2 min recovery are:

- Left ventricular biplane ejection fraction (LVEF %)
- Left ventricular peak systolic mean longitudinal strain (LV PSMLS %)
- Left ventricular peak systolic mean circumferential strain (LV PSMCS %),
- Diastolic ratio (MV E / LV E' cm/s)

The main MRI parameters of interest at rest are:

- LVEF %

- Right ventricular ejection fraction (RVEF %)

All these parameters will be analysed formally using mixed linear regression models to compare bezafibrate against placebo, in the same way, using the same methods as the primary analysis of peak VO_2 above.

Other data collected relating to cardiac function will be summarised descriptively using means and standard deviations (SDs) (or medians and interquartile range (IQR) depending on the distribution) or counts and percentages (where appropriate) by treatment, period and overall and will include:

Cardiac function at rest:

- LV function
  - Fraction shortening (Teichholz) (%)
  - Biplane ejection fraction (%)
  - Myocardial performance index
  - Mitral annular plane systolic excursion (MAPSE) – lateral and septal (mm)
- LV Tissue Doppler
  - S', E' and A' wave velocity (cm/s)
  - IVA (m/s^2)
  - E/E'
  - Mean systolic longitudinal strain (%) and strain rate
  - Lateral basal, mid and apical (%)
  - Septal basal, mid and apical (%)
  - Mean systolic circumferential strain (%) and strain rate
  - Anterior septal, anterior, lateral, posterior, inferior, septal (%)
  - E and A wave velocity (cm/s)
  - Decelation time (ms)
  - E/A
  - IVS S', E' and A' wave velocity (cm/s)
  - IVS IVA (m/s^2)
  - 3D mean systolic strain
  - Wall motion abnormalities (Yes, No) – including description
- RV function
  - Wall motion abnormalities (Yes, No) – including description
  - TR peak velocity (m/s) (if given)
  - TAPSE (mm)
  - Myocardial performance index
  - Fractional area of change (%)
  - Mean systolic longitudinal strain (%) and strain rate
  - Lateral basal, mid, and apical (%)
- RV Tissue Doppler (pulsed wave)
  - S', E' and A' wave velocity (cm/s)
  - IVA (m/s^2)
  - E/E'
  - E and A wave velocity (cm/s)

Cardiac function during exercise will also be summarised descriptively using means and SDs (or medians and IQRs) or counts and percentages (where appropriate) by treatment, period and overall and will include:

- Exercise history
  - Exercise (hr/week)
  - Self-assessment of exercise capacity (1 = Low, 10 = Elite)

  - Motivation (Low, Average, High)

- Exercise test
  The following will be taken at: baseline (pre-exercise); at 20W increments starting at 0W until 160W; and in recovery (2 and 6 mins post-exercise)
  - Heart rate (bpm)
  - Systolic and diastolic blood pressure (mmHg)
  - Peripheral capillary oxygen saturation (SpO_2) (%)
  - Comments on symptoms and ECG
  - Near-infrared spectroscopy (NIRS) (%)

  The following will also form part of the exercise test:
  - Exercise duration (m:s)
  - Achieved work rate
  - Cause and comments on cessation
  - Perceived exercise effort – Borg scale at end of exercise (1-10)
  - Peak heart rate (bpm)
  - Systolic and diastolic blood pressure at rest and peak (mmHg)

- Exercise ECHO
  The following will be taken at: baseline (pre-exercise); at 20W increments starting at 0W until 160W; and in recovery (2 and 6 mins post-exercise)
  - MV E cm/s
  - LV PSMLS %
  - LV PSMCS %
  - LV S' cm/s
  - LV E' cm/s
  - RV PSMLS %
  - TR m/s
  - RV S' cm/s
  - RV E' cm/s

  The following will also form part of the exercise ECHO:
  - Anaerobic threshold (%)
  - O2 pulse (ml/beat)
  - RER
  - METs

- VO_2 measurement at baseline (pre-exercise) and at recovery (2 and 6 mins post-exercise) (ml/kg/min)

Cardiac function by ECG will be summarised descriptively using means and SDs (or medians and IQRs) or counts and percentages (where appropriate) by treatment, period and overall. ECG at rest will include:

- Heart rate (bpm)
- Baseline rhythm, status of the following:
  - Sinus rhythm
  - Heart block
  - Atrial fibrillation/flutter

    - Paced
- P wave normal
- P-axis (°)
- PR-interval (ms)
- QRS-axis (°)
- QRS-duration (ms)
- Left ventricular hypertrophy
- Right ventricular hypertrophy
- ST-segments
- ST depression
- TWI
- T axis (°)
- QTc (ms)

ECG during exercise will report new rhythm (sinus rhythm, heart block, atrial fibrillation and pacing occurrence), if changed.

Cardiac MRI data will also be summarised descriptively using line listings, means and SDs (or medians and IQRs) or counts and percentages (where appropriate) by treatment, period and overall, and will include for both left and right ventricular function in turn:

- Existence of wall type abnormalities, and if so, what type and where
- Structure
- Absolute and indexed ventricular function:
    - Ejection fraction (%)
    - End diastolic volume (mL/m^2)
    - End systolic volume (mL/m^2)
    - Stroke volume (mL)
    - Cardiac output (L/min)
    - Cardiac index (L/min/m^2)
    - Mass (g/m^2)

Lymphocyte mitochondria

Lymphocyte mitochondria will be summarised descriptively using means and SDs (or medians and IQRs) by treatment, period and overall and will include:

- Mitochondrial size
- Numbers of mitochondria per lymphocyte
- Total area of mitochondria per lymphocyte
- Area of mitochondria as proportion of cytoplasm
- Mitochondria function and cristae organisation in lymphocytes

Arrhythmia profile

Arrhythmia profile will be summarised descriptively using counts and percentages by treatment, period and overall and will include:

- Sinus rhythm (Yes, No) at rest and during exercise

4.6 General content

4.6.1 Participant flow

The CONSORT [2] flow diagram for crossover trials will be used to descriptively summarise the course of participants through screening until follow-up throughout the course of the study, this will include the number of:

- Patients fulfilling the inclusion criteria
- Eligible patients
- Patients given/sent a patient information leaflet (PIL)
- Patients approached
- Patients giving consent to participate
- Participants randomised
- Participants allocated to each sequence
- Participants who receive at least one dose of trial drug
- Participants who reach wash-out period
- Participants who reach end of second phase
- Participants who withdraw at any time

Protocol deviations defined in section 3.3.2, and confirmed serious breaches of good clinical practice (GCP) will be summarised descriptively by sequence, including violations of eligibility criteria on entry into the study. The number of withdrawals of consent to the study will be presented, along with reasons for withdrawal. Any notes to file reported for each participant will be presented as a line listing.

4.6.2 Baseline data

Baseline characteristics to be summarised descriptively using means and SDs (or medians and IQRs) or counts and percentages (where appropriate) by sequence and overall will include:

- Patient type (transplant, non-transplant)
- Age (and age group: <11 years, 11-15 years, 16+ years)
- Weight (kg), height (cm) and body surface area (BSA) (m^2)
- Resting measurements:
    - O2 saturations (%)
    - Systolic and diastolic blood pressure (mmHg)
    - Heart rate (bpm)
    - Respiratory rate (breaths per min)
- Heart sounds normal
- Pulses regular
- Signs of heart failure:
    - Respiratory distress
    - Hepatomegaly
    - Pulmonary auscultation findings
    - Ascites
    - Pitting oedema
- History of the following in the last 4 months, and whether related to exercise:
    - Fainting
    - Dizziness
    - Palpitations
    - Chest pain or tightness
    - Unexpected decrease in exercise capacity or fitness level

Baseline medication history, including dose, unit and frequency of drugs received, will be summarised descriptively using counts and percentages by sequence and overall and will include:

- Cardiac drug usage:
  - Amlodipine
  - Aspirin
  - Atorvastatin
  - Bisoprolol
  - Captopril
  - Carvedilol
  - Digoxin
  - Enalapril
  - Furosemide
  - Lisinopril
  - Pravastatin
  - Propanolol
  - Ramipril
  - Spironolactone
- General supportive care drug use:
  - Citalopram
  - Folic acid
  - Forceval
  - Gaviscon
  - Glycozade
  - Lactulose
  - Lansoprazole
  - Movical
  - Multivitamins
  - Omeprazole
  - Panothenate
  - Pizotifen
  - Ranitidine
  - Seravit
  - Sertroline
  - Sytron
  - Thiamine
- Transplant-related drugs:
  - Ciclosporin
  - Mycophenolate mofetil (MMF)
  - Prednisolone
  - Sirolimus
  - Tacrolimus
- Infection prophylaxis drugs:
  - Amoxicillin
  - Azithromycin
  - Cotrimoxazole
  - Granulocyte-colony stimulating factor (G-CSF)
  - Penicillin
- Other drugs

4.6.3 Trial medication data

The dose received by each participant will vary according to the participant's age in the following way:

- Children aged 6-9 years: commence on 100mg once daily for the first month and if well tolerated increase to 100mg twice daily for the remaining 3 months
- Children aged 10-17 years: commend on 200mg once daily for the first month and if well tolerated increase to 200mg twice daily for the remaining 3 months
- Adults aged 18+ years: 200mg twice daily

Dose received by each participant, in each month of each period and in total, will be summarised descriptively using means and SDs (or medians IQRs), along with details of, and reasons for, any dose omissions, by treatment, period and overall.

4.6.4 Other medication data

Any other medications the participant receives during the study will be summarised descriptively using means and SDs (or medians and IQRs) by sequence, treatment, period and overall.

4.6.5 Research blood tests

All research blood tests will be summarised descriptively using means and SDs (or medians and IQRs) or counts and percentages (for eGFR) by treatment and period, with the exception of neutrophil counts, and plasma arginine and cysteine levels, which will be summarised as part of the secondary outcomes. The blood tests to be summarised will be:

- Creatine kinase (U/L)
- Hb (g/L)
- WBC (109/L)
- Platelets
- Lymphocytes
- Monocytes
- Creatinine
- eGFR (mL/min) (<90; >90)
- Urea (mmol/L)
- Sodium (mmol/L)
- Potassium (mmol/L)
- Bircarbonate (mEq/L)
- Calcium (mmol/L)
- Bilirubin
- Alkaline phosphatase (IU/L)
- Alanine aminotransferase (U/L)
- Total protein (g/L)
- Albumin (g/L)
- Brain natriuretic peptide
- Total cholesterol
- High-density lipoprotein (HDL) cholesterol
- Triglycerides

4.6.6 Harms

Harms will be summarised descriptively using concordant and discordant pairs, and line listings for all adverse events (AEs) and serious adverse events (SAEs) where events will be presented

using the safety population according to the treatment the participant received in the relevant period. Line listings of all reported events will include (where appropriate):

- Treatment randomised to in period
- Treatment received in period (if different)
- Date of randomisation
- Date of onset
- Event name
- Expectedness

The additional information will be presented forserious events (where appropriate):

- SAE/SAR/SUSAR full description
- MedDRA system organ class
- SAE/SAR/SUSAR classification (seriousness)
- Relatedness
- Maximum intensity
- Time of onset
- Outcome
- Date of recovery
- Duration of the event
- Unblinding information

Safety blood tests will also be taken in months 1-3, during the wash-out period, and in months 6-8. The results from these tests will be summarised descriptively using means and SDs (or medians and IQRs) by treatment received and overall, and will include:

- Creatinine kinase (U/L)
- Hb (g/L)
- WBC (10^9/L)
- Platelets (10^9/L)
- Neutrophils (10^9/L)
- Creatinine (µmol/L) (also collected at week 2 in both periods)
- Urea (mmol/L)
- Sodium (mmol/L)
- Potassium (mmol/L)
- Bicarbonate (mEg/L)
- Calcium (mmol/L)
- Bilirubin (µmol/L)
- Alkaline phosphatase (IU/L)
- Total protein (g/L)
- Albumin (g/L)
- Total cholesterol
- LDL
- Triglycerides

4.7 Missing data and outliers

A thorough data cleaning process will be carried out and attempts will be made to obtain any missing data by chasing until it is either received, confirmed as not available, or the study is at the analysis stage. Were data is unobtainable, all summaries will indicate how many missing results there are, by sequence, where any imbalances can be explored. Where data points are identified

as possible outliers both statistically and clinically, and are considerable in number, sensitivity analyses may be considered for the primary outcomes.

5. References

1. Schulz, K.F., et al., *Consort 2010 statement: Updated guidelines for reporting parallel group randomized trials.* Annals of Internal Medicine, 2010. **152**(11): p. 726-732.
2. James W. Varni, P.D., *Scaling and Scoring of the Pediatric Quality of Life Inventory™*

PedsQL. Version 17: May 2017.

6. Revision history

Version 1 of the SAP should be signed off by relevant personnel before any data analysis is carried out. If changes need to be made to v1.0 before this time, possibly due to emerging methodologies, these changes should be documented in Table 1 below, with new version number, date and a summary of the changes with justification(s). If any changes to the methodologies are required after data analysis has begun, these should be documented in the final analysis report in a chronological manner, documenting all decisions made and their justification(s).

Table 1 SAP revision history

Version number	Revision date	Justification for revision