A randomised, double-blind, placebo-controlled, phase 2 evaluation of the efficacy and mechanism of trientine in patients with hypertrophic cardiomyopathy (TEMPEST)

TEMPEST Protocol v2.0 09/07/2020

Study Sponsor:

Manchester University NHS Foundation Trust,

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PROTOCOL APPROVAL

I, the undersigned, hereby approve this clinical study protocol:

Authorised by Chief Investigator:

Signature: _____ **Date:** _____

Dr Chris Miller
Cardiology Department, Wythenshawe Hospital,
Manchester University NHS Foundation Trust,
Manchester, M23 9LT

I, the undersigned, hereby approve this clinical study protocol:

Authorised on behalf of Sponsor:

Signature: _____ **Date:** _____

Dr Lynne Webster, Head of Research Office
Head of Research Office, Research office,
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I, the undersigned, hereby approve this clinical study protocol:

Authorised on behalf of the Lead Statistician:

Signature: _____ **Date:** _____

Dr Susanna Dodd
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General Information

This document describes the TEMPEST trial and provides information about procedures for entering patients into it. The protocol should not be used as an aide-memoir or guide for the treatment of other patients; every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to the registered investigators in the trial, but centres entering patients for the first time are advised to contact the coordinating centre (Liverpool Clinical Trials Centre (LCTC)) to confirm they have the most up to date version. Clinical problems relating to this trial should be referred to the Chief Investigator (CI), Dr Chris Miller, via the LCTC.

This protocol defines the participant characteristics required for study entry and the schedule of treatment and follow-up. Participant recruitment will be undertaken in compliance with this document and applicable regulatory and governance requirements, and waivers to authorise non-compliance are not permitted.

Incidence of protocol non-compliance, whether reported prospectively (e.g. where a treatment cannot be administered on a scheduled date because of public holidays) or retrospectively noted (e.g. as a result of central monitoring) are recorded as protocol deviations, the incidence of which are monitored and reported to trial oversight committees.

The template content structure is consistent with the SPIRIT (Standard Protocol Item: Recommendations for Interventional Trials 2013) and has regard for the Health Research Authority (HRA) guidance. Regulatory and ethical compliance information is located in section 12.

Relationship Statements

Roles and responsibilities are fully described in section 15.

Manchester University NHS Foundation Trust is the Sponsoring organisation and will formally delegate specific sponsoring roles to the CI and Clinical Trials Unit, but remains legally responsible for the trial.

Clinical Trials Unit: The LCTC at the University of Liverpool in collaboration with the CI, Dr Chris Miller, will have overall management responsibility for the trial from the LCTC's perspective and will be responsible for the co-ordination of centres.

The LCTC as part of the Liverpool Clinical Trials Collaborative has achieved full registration by the UK Clinical Research Collaboration (www.ukcrc.org) as their standards and systems were assessed by an international review panel as reaching the highest quality. The LCTC has a diverse trial portfolio underpinned by methodological rigour, a GCP compliant data management system, and core standard operating procedures (SOPs).

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Contact Details: Individuals

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In cases where the CI is unavailable to respond to urgent queries the following individual/s will act as cover:

Medical Expert who will Advise on Protocol Related Clinical Queries:	Medical Expert who will Evaluate SAE Reports:
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Additional Contacts:

The contact details of other individuals involved in the trial are detailed in documents supplementary to the protocol and stored in the Trial Master File:

Contact	Document Title
Trial Management Group (TMG) Trial Steering Committee (TSC) Independent Data and Safety Monitoring Committee (IDSMC)	TEMPEST Trial Oversight Committee Membership
Principal Investigators	TEMPEST Participating Centres

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Glossary

AE	Adverse Event
AR	Adverse Reaction
ATP	Adenosine triphosphate
BMI	Body mass index
BSA	Body surface area
CI	Chief Investigator
CMR	Cardiovascular magnetic resonance
CPET	Cardiopulmonary exercise testing
CRF	Case Report Form
CT	Clinical Trials
CTA	Clinical Trials Authorisation
CTIMP	Clinical Trials of an Investigational Medicinal Product
CTU	Clinical Trials Unit
ECG	Electrocardiogram
ECM	Extracellular matrix
eCRF	Electronic Case Report Form
ECV	Extracellular volume
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
EUDRACT	European Clinical Trials Database
GCP	Good Clinical Practice
GP	General Practitioner
HCM	Hypertrophic cardiomyopathy
HRA	Health Research Authority
HS troponin	High sensitivity troponin
IB	Investigator's Brochure
IDSMC	Independent Data and Safety and Monitoring Committee
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IS	Information System
ISF	Investigator Site File
ITT	Intention to treat
IRB	Independent Review Board
JVP	Jugular venous pressure
KCCQ	Kansas City cardiomyopathy questionnaire
LA	Left atrium
LCTC	Liverpool Clinical Trials Centre
LV	Left ventricle
LVEF	Left ventricular ejection fraction
LVH	Left ventricular hypertrophy
LVMi	Left ventricular mass indexed to body surface area
MC CTU	Medicines for Children Clinical Trials Unit
MFT	Manchester University NHS Foundation Trust
MHRA	Medicines and Health Care Products Regulatory Agency
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NHS	National Health Service

NRES	National Research Ethics Service
NIHR CRN	National Institute for Health Research Clinical Research Network
NYHA	New York Heart Association
31P	31Phosphorus
PCr	Phosphocreatine
PCr/ATP	Phosphocreatine to adenosine triphosphate ratio
PI	Principal Investigator
PISC	Participant Information Sheet and Consent form
R&D	Research & Development
REC	Research Ethics Committee
RN	Research Nurse (Registered)
RSI	Reference Safety Information
RSO	Research Support Office
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SD	Standard deviation
SDV	Source Data Verification
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SSAR	Suspected Serious Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TSC	Trial Steering Committee
UAR	Unexpected Adverse Reaction
ULN	Upper limit of normal

2 PROTOCOL SUMMARY

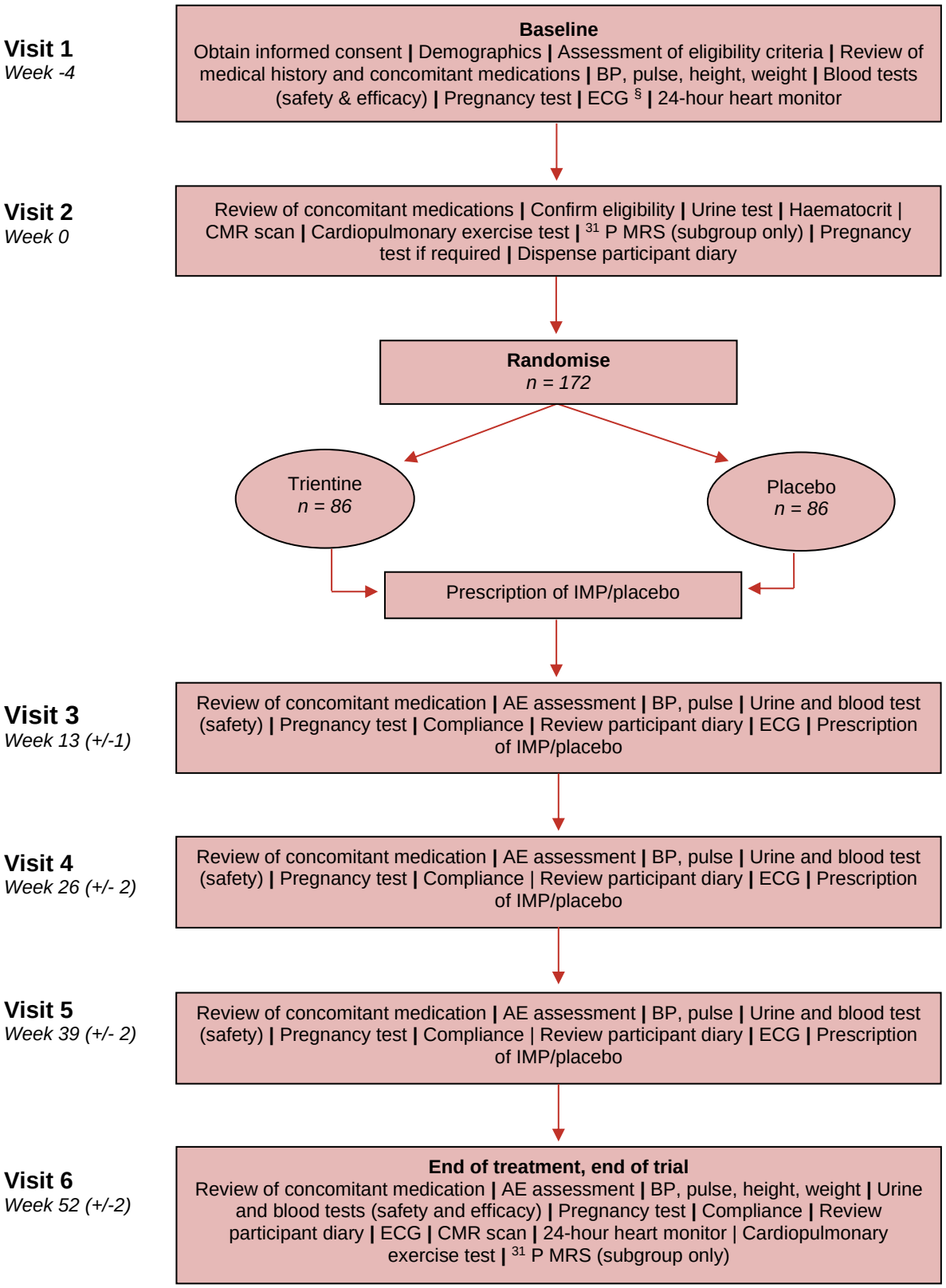
Full Title	A randomised, double-blind, placebo-controlled, phase 2 evaluation of the efficacy and mechanism of trientine in patients with hypertrophic cardiomyopathy
Acronym	TEMPEST
Phase	II
Sample size	172
Number of participants undergoing ^{31}Phosphorus magnetic resonance spectroscopy (subgroup)	84 (42 in each treatment group) from the Oxford and Manchester sites
Main Inclusion Criteria	1. Written informed consent. 2. Age 18-70 inclusive. 3. Hypertrophic cardiomyopathy (HCM), as defined by the European Society of Cardiology HCM guidelines as: "a wall thickness ≥15 mm in one or more LV myocardial segments that is not explained solely by loading conditions". The same definition is applied to first-degree relatives of patients with HCM i.e. all participants are required to have a LV wall thickness ≥15 mm. Wall thickness is as measured on the most recent cardiovascular magnetic resonance (CMR) scan performed prior to the baseline visit. If CMR has not been performed previously, wall thickness measurement should be taken from the most recent echocardiogram performed prior to the baseline visit. (It is recognised that in the European Society of Cardiology guidelines a clinical diagnosis of HCM in first-degree relatives requires a wall thickness that is less than this value, however ≥15 mm is applied here in order to ensure that all participants have an unequivocal phenotype). 4. New York Heart Association class I, II or III at the most recent clinical assessment performed prior to the baseline visit.
Main Exclusion Criteria	1. Previous or planned septal reduction therapy. 2. Previously documented myocardial infarction or severe coronary artery disease. 3. Uncontrolled hypertension, defined as a systolic blood pressure of >180mmHg or a diastolic blood pressure of > 100mmHg at Visit 1. 4. Known LV EF < 50%, as measured on the most recent CMR scan performed prior to the baseline visit. If CMR has not been performed previously, the most recent echocardiogram performed prior to the baseline visit should be used. 5. Previously documented persistent atrial fibrillation. 6. Anaemia, defined as haemoglobin being below the local site normal reference range, at Visit 1. 7. Iron deficiency, defined as serum iron being below the local site normal reference range, at Visit 1.

	8. Copper deficiency, defined as serum copper being below the normal reference range, at Visit 1. 9. Pacemaker or implantable cardioverter defibrillator. 10. Known severe valvular heart disease, as demonstrated on the most recent heart imaging performed prior to the baseline visit. 11. Previously documented other cardiomyopathic cause of myocardial hypertrophy (e.g. amyloidosis, Fabry disease, mitochondrial disease). 12. History of hypersensitivity to any of the components of the investigational medicinal product (IMP). 13. Known contraindication to MRI scanning. 14. Pregnancy, lactation or planning pregnancy. Women of childbearing capacity are required to have a negative serum pregnancy test before treatment, must agree to pregnancy tests at study visits as defined in the Section 8 and must agree to maintain highly effective contraception as defined in Section 8 during the study. 15. Any medical condition, which in the opinion of the Investigator, may place the patient at higher risk from his/her participation in the study, or is likely to prevent the patient from complying with the requirements of the study or completing the study.	
Study Centres and Distribution:	NHS Hospitals and non-NHS sites in the UK	
Patient Study Duration	52 weeks from randomisation	
Intervention	**Active intervention:** Trientine dihydrochloride. Taken orally as two Cufence 200mg capsules two times per day. (Total daily dose Cufence 800mg). **Control intervention:** Matching Placebo. Taken orally as two capsules two times per day.	
Overall objectives	To evaluate the clinical efficacy and mechanism of action of trientine in hypertrophic cardiomyopathy.	
	Objectives	**Outcome Measures**
Primary efficacy objective	To determine whether trientine compared to placebo leads to regression of LV hypertrophy.	Change in left ventricular mass indexed to body surface area (LVMi)
Secondary efficacy objectives	To test whether trientine compared to placebo: 1. Increases urinary copper excretion 2. Improves exercise capacity 3. Reduces arrhythmia burden 4. Reduces cardiomyocyte injury	1. Cumulative urine copper excretion, measured using urinary copper. 2. Change in exercise capacity, measured using cardiopulmonary exercise testing (CPET). 3. Change in number of non-sinus supraventricular heart beats, presence and amount

	5. Improves LV contractile function 6. Improves left atrial structure and function	of atrial fibrillation, number of ventricular-origin beats and presence and amount of non-sustained ventricular tachycardia, in 24 hours, measured using ambulatory heart monitoring. 4. Change in circulating high sensitivity troponin. 5. Change in LV global longitudinal strain and strain rate, wall thickness, mass, volumes and ejection fraction (EF) measured using CMR. 6. Change in atrial volume and function, measured using CMR.
Mechanistic objectives	To understand how trientine causes a reduction in LV hypertrophy the study will determine whether: 1. Trientine, compared to placebo, leads to cellular or extracellular mass regression. 2. Trientine, compared to placebo, leads to an improvement in myocardial energetics. 3. LV hypertrophy regression is mediated by cellular regression, extracellular regression or improved myocardial energetics, which are in turn determined by copper excretion.	1. Change in LV myocardial cellular mass, measured using CMR 2. Change in LV myocardial extracellular mass, measured using CMR 3. Change in LV myocardial extracellular volume, measured using CMR 4. Change in LV late gadolinium enhancement, measured using CMR 5. Change in PCr/ATP ratio, measured using 31P MRS (sub-group)

Protocol Summary - continued

Schematic of Study Design:



§May be performed at Visit 1 or Visit 2 but must be done before randomisation.
Pregnancy test applies to females of childbearing capacity only.
At Visit 2, female participants of childbearing capacity will be asked if there is a chance that they could have become pregnant since Visit 1. If the participant confirms that there is a chance, then a pregnancy test will be performed.

3 INTRODUCTION

3.1 Background

3.1.1 Hypertrophic cardiomyopathy (HCM)

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiovascular disorder, with a contemporary estimated prevalence of around 1 in 200 people, meaning that more than 3.5 million people in Europe are affected.[1]

HCM is a mono-genetically determined heart muscle disease most often caused by mutations in one of several contractile protein genes. It is characterised by left ventricular (LV) myocardial hypertrophy, disorganised cardiomyocytes (disarray) and fibrosis. Mean age at diagnosis is around 40.[2]

Clinical manifestations of HCM are variable but can be devastating. Two thirds of patients have symptoms at diagnosis, including breathlessness, chest pain, effort intolerance, palpitations and blackouts.[3] One in twenty patients develop advanced heart failure within 5–8 years, and 1:25 patients have a stroke or peripheral embolism each year.[4-6] Importantly, 1% of patients die suddenly each year due to ventricular arrhythmias thought to be precipitated in particular by myocardial fibrosis; indeed HCM is the leading cause of sudden death in people under 35.[6] Clinical manifestations are particularly deleterious given the young age of patients.[7]

There is no disease modifying treatment for HCM. Treatment currently comprises therapies to palliate symptoms and defibrillators to prevent sudden death in patients deemed high risk.

Patients and the cardiovascular field have identified an urgent need for clinical studies of drug therapies that target HCM pathophysiological mechanisms in order to improve the lives of the millions of people living with the condition.[8, 9] For example, the National Institutes of Health have identified a “critical need” to undertake clinical studies to determine if novel (or currently available) drug therapies can target putative pathways of HCM disease expression and, thereby, improve on the natural history of patients”.[8]

3.1.2 Rationale for trientine in HCM

Trientine dihydrochloride is a highly selective copper II chelator already licensed for use in Wilson disease, a genetic disorder of copper excretion. Patients with Wilson disease exhibit a cardiac phenotype that mimics HCM.

Type II diabetes is also associated with LV hypertrophy and abnormal copper homeostasis.[10] In preclinical diabetic models, trientine is associated with reduced LV hypertrophy and fibrosis and improved LV function, cardiomyocyte structure and organisation of muscle fibres.[11-15]

In a placebo-controlled RCT in patients with type II diabetes and LV hypertrophy, 12 months of trientine dihydrochloride 1200mg/day was associated with a significant reduction in LV hypertrophy (change in LV mass indexed to body surface area (LVMi) with trientine: -10.6±7.6g/m2 v placebo -0.1±9.8g/m2; $p<0.01$) without changes in blood pressure or glucose.[16] Cumulative urine copper excretion with trientine treatment independently associated with decrease in LVMi. Furthermore, cumulative urine copper excretion with trientine treatment was higher in patients with higher baseline LV mass.

As such, trientine appears to modulate key pathological features of HCM i.e. LV hypertrophy, myocyte disarray and fibrosis.

HCM is associated with altered copper homeostasis; specifically, HCM is associated with elevated serum copper and caeruloplasmin in comparison to matched healthy volunteers.[17]

In an open-label pilot study in patients with HCM, 6 months of trientine dihydrochloride at a dose of 1200mg/day was associated with a non-significant reduction in LVMi, significant improvements in LV function and left atrial function, and a strong trend towards decreased myocardial fibrosis.[18] The results are encouraging despite treatment duration being only 6 months, compared to 12 months in the diabetes trial, in which LVMi reduction at 12 months was double that at 6 months

3.1.3 Potential mechanism of action of trientine in HCM

Unbound/loosely bound tissue copper II ions are powerful catalysts of reactive oxygen species and oxidative stress, and inhibitors of enzymatic antioxidants such as extracellular superoxide dismutase.

Trientine has a range of actions thought to result from its removal of copper II ions from tissue. Trientine is associated with restoration of mitochondrial ultrastructure and normalisation of myocardial expression and enzymatic activity of proteins involved with energy metabolism, components of the mitochondrial respiratory chain and enzymes involved in fatty acid oxidation.[11, 19] This is highly relevant to HCM because energy depletion is widely hypothesised to be a mechanism by which gene mutations lead to the phenotype.[20] Significantly impaired myocardial energetics are observed in HCM sarcomeric mutation carriers before they develop LV hypertrophy, suggesting energy deficiency is a primary event (reduced phosphocreatine (PCr) to adenosine triphosphate (ATP) ratio, measured using ^{31}phosphorus magnetic resonance spectroscopy (^{31}P MRS)).[21] Also, impaired myocardial energetics in HCM are associated with progressive myocardial fibrosis.[22] Furthermore, inherited defects in mitochondrial energy production and fatty-acid oxidation lead to phenotypes mimicking HCM.[23]

Trientine also normalises extracellular superoxide dismutase, which inhibits reactive oxygen species-mediated tissue growth factor-B activation and reverses myocardial fibrosis.[12, 14]

3.2 Risk and Benefits

The recruiting clinician will discuss the potential risks and benefits with patients prior to trial entry and they will be outlined in the participant information sheet and consent form (PISC).

3.2.1 Potential Risks

Trientine has been used in Wilson disease for more than 30 years. It is safe and well tolerated.

The Summary of Product Characteristics (SmPC) states nausea can commonly occur on initial treatment and occasionally skin rash can occur.

The SmPC states that duodenitis and severe colitis have been reported. The frequency is given as “Not known”. Specifically, there have been 2 case reports of colitis/duodenitis. In one of these cases the patient had “colitis-irritable gut” before starting trientine. In one case the colitis resolved with dose reduction and in the other it resolved on discontinuation.[27]

The SmPC states it is uncommonly associated with anaemia, aplastic anaemia and sideroblastic anaemia. There have been 2 case reports of sideroblastic anaemia, both of which occurred with higher doses of trientine than which will be used in the current trial (double the dose in one case, almost double the dose in the other case), and in both cases it resolved after reducing the dose.[24, 25] There has been one case report of leukopenia that resolved with dose reduction/treatment holiday (the paper does not specifically state which).[26] The SmPC states trientine has been found to reduce serum iron levels, possibly by reducing its absorption.

In the aforementioned pilot study, trientine was safe and well tolerated. Four of 20 patients withdrew due to adverse events (1 migraine [pre-existing], 1 arthralgia [pre-existing], 1 abdominal bloating and 1 oesophageal ulcer [unrelated to trientine]). Of the fifteen patients who completed the study, two took a reduced dose of trientine dihydrochloride (600mg/day) for the final 2 months due to gastrointestinal symptoms. There were no changes in serum iron, haemoglobin or white cell count with trientine.

3.2.2 Potential Benefits

Participants may have a more detailed cardiovascular assessment than they usually would. They will receive closer follow-up than usual care, and have more access to heart specialists than usual. Participants will also help to determine whether trientine will be of benefit to patients HCM, and contribute to a better understanding of HCM.

3.3 Hypotheses

3.3.1 Efficacy hypothesis

Trientine will reduce LV mass, which will be associated with improved exercise capacity, reduced arrhythmia burden and improved cardiac function.

3.3.2 Mechanistic hypothesis

The reduction in LV mass will be mediated by a reduction in myocardial cellular mass and fibrosis and improved myocardial energetics, which will be determined by increased copper excretion.

3.4 Objectives

The aim of this trial is to evaluate the clinical efficacy and mechanism of action of trientine in HCM.

3.4.1 Efficacy Objectives

3.4.1.1 Primary efficacy objective

To determine whether trientine compared to placebo leads to regression of LV hypertrophy.

3.4.1.2 Secondary efficacy objectives

To test whether trientine compared to placebo:

1. Increases urinary copper excretion
2. Improves exercise capacity
3. Reduces arrhythmia burden
4. Reduces cardiomyocyte injury
5. Improves LV contractile function
6. Reduces left ventricular outflow tract gradient
7. Improves left atrial structure and function

3.4.2 Mechanistic objectives

To understand how trientine causes a reduction in LV hypertrophy the study will determine whether:

1. Trientine, compared to placebo, leads to cellular or extracellular mass regression.
2. Trientine, compared to placebo, leads to an improvement in myocardial energetics.
3. LV hypertrophy regression is mediated by cellular regression, extracellular regression or improved myocardial energetics, which are in turn determined by copper excretion.

3.4.3 Safety objectives

To evaluate the safety of trientine in HCM.

3.4.4 Other objectives

1. To test whether the effect of trientine varies according to genotype.
2. To record screening and recruitment data to inform a subsequent phase III study.
3. To collect clinical (phase III) endpoints that could potentially be rolled forward to a future phase III trial.

4. To obtain consent from participants to enable long term follow-up using routinely collected clinical data with appropriate linkage.

3.5 Outcome measures

3.5.1 Efficacy outcome measures

3.5.1.1 Primary

Change in LVMi (g/m2), measured using CMR, from baseline to week 52.

3.5.1.2 Secondary

All assessed from baseline to week 52, except urine copper excretion, which is assessed from baseline to weeks 13, 26, 39 and 52.

1. Cumulative urine copper excretion, measured using urinary copper.
2. Change in exercise capacity, measured using cardiopulmonary exercise testing (CPET).
3. Change in number of non-sinus supraventricular heart beats, presence and amount of atrial fibrillation, number of ventricular-origin beats and presence and amount of non-sustained ventricular tachycardia, in 24 hours, measured using ambulatory heart monitoring.
4. Change in circulating high sensitivity troponin.
5. Change in LV global longitudinal strain and strain rate, wall thickness, mass, volumes and ejection fraction (EF) measured using CMR.
6. Change in peak left ventricular outflow tract gradient, measured using CMR.
7. Change in atrial volume and function, measured using CMR.

3.5.2 Mechanistic outcome measures

All assessed from baseline to week 52.

1. Change in LV myocardial cellular mass, measured using CMR
2. Change in LV myocardial extracellular mass, measured using CMR
3. Change in LV myocardial extracellular volume, measured using CMR
4. Change in LV late gadolinium enhancement, measured using CMR
5. Change in PCr/ATP ratio, measured using 31P MRS (sub-group)

3.5.3 Safety outcome measures

1. Treatment-emergent AEs, SAEs, SARs, SUSARs.
2. Treatment-emergent changes in laboratory investigations, including haemoglobin, leukocyte count, platelet count, serum iron studies, serum copper studies, and renal function.
3. Treatment-emergent changes in pulse and blood pressure.
4. Treatment-emergent changes in electrocardiogram (ECG).

3.5.4 Other outcome measures

1. Screening and recruitment data will be collected in order to inform a subsequent phase III study.
2. Clinical (phase III) endpoints: death from cardiovascular causes, appropriate defibrillator discharge/aborted sudden death, heart transplant, progressive heart failure symptoms, defined as progression from New York Heart Association class I/II to III/IV, new atrial fibrillation and septal reduction therapy.

4 TRIAL DESIGN

The trial is a phase II double-blind, parallel group, 1:1 randomised placebo-controlled multi-centre clinical trial of trientine versus placebo in 172 patients with HCM. A subgroup of 84 patients (aiming for 42 in each treatment group) will undergo ^{31}P MRS as part of the mechanistic evaluation.

5 STUDY SETTING AND SELECTION OF CENTRES

The TEMPEST trial will take place in NHS hospitals and non-NHS sites in the UK.

5.1 Selection of Centres/Clinicians

Criteria for the selection of centres will be determined by the TMG and will be described in the supplementary document 'Site Suitability Assessment'.

Initiation of centres will be undertaken in compliance with LCTC SOPs; Centres fulfilling the criteria will be selected to be recruitment centres for the TEMPEST trial and will be opened to recruitment upon successful completion of all global (e.g. Multicentre Research Ethics Committee (MREC) and Medicines and Health Care Products Regulatory Agency (MHRA)) and study-specific conditions (e.g. site personnel training requirements) and once all necessary documents have been returned to the LCTC as detailed in the trial 'greenlight' checklist.

Participating centres will be listed in the 'TEMPEST Participating Centres' log, maintained separately to the protocol.

6 STUDY POPULATION

The TEMPEST trial aims to recruit 172 patients based on sample size calculations described in Section 11.2. All patients must provide written, informed consent before any study procedures occur (see Section 7.2 for more information regarding informed consent processes) and must meet all eligibility criteria as described below.

6.1 Inclusion Criteria

Patients eligible for inclusion in this study should fulfil all of the following criteria:

1. Written informed consent.
2. Age 18-70 inclusive.
3. HCM, as defined by the European Society of Cardiology HCM guidelines as: "a wall thickness ≥15 mm in one or more LV myocardial segments that is not explained solely by loading conditions".[6] The same definition is applied to first-degree relatives of patients with HCM i.e. all participants are required to have a LV wall thickness ≥15 mm. Wall thickness is as measured on the most recent CMR scan performed prior to the baseline visit. If CMR has not been performed previously, wall thickness measurement should be taken from the most recent echocardiogram performed prior to the baseline visit.

   (It is recognised that in the European Society of Cardiology guidelines a clinical diagnosis of HCM in first-degree relatives requires a wall thickness that is less than this value, however ≥15 mm is applied here in order to ensure that all participants have an unequivocal phenotype).
4. New York Heart Association class I, II or III at the most recent clinical assessment performed prior to the baseline visit.

6.2 Exclusion Criteria

Any patient meeting any of the criteria listed below at baseline will be excluded from study participation:

1. Previous or planned septal reduction therapy.
2. Previously documented myocardial infarction or severe coronary artery disease.
3. Uncontrolled hypertension, defined as a systolic blood pressure of >180mmHg or a diastolic blood pressure of > 100mmHg at Visit 1.
4. Known LV EF < 50%, as measured on the most recent CMR scan performed prior to the baseline visit. If CMR has not been performed previously, the most recent echocardiogram performed prior to the baseline visit should be used.
5. Previously documented persistent atrial fibrillation.
6. Anaemia, defined as haemoglobin being below the local site normal reference range, at Visit 1.
7. Iron deficiency, defined as serum iron being below the local site normal reference range, at Visit 1.
8. Copper deficiency, defined as serum copper being below the normal reference range, at Visit 1.
9. Pacemaker or implantable cardioverter defibrillator
10. Known severe valvular heart disease, as demonstrated on the most recent heart imaging performed prior to the baseline visit.
11. Previously documented other cardiomyopathic cause of myocardial hypertrophy (e.g. amyloidosis, Fabry disease, mitochondrial disease).
12. History of hypersensitivity to any of the components of the investigational medicinal product (IMP).
13. Known contraindication to MRI scanning.

14. Pregnancy, lactation or planning pregnancy. Women of childbearing capacity are required to have a negative serum pregnancy test before treatment, must agree to pregnancy tests at study visits as defined in the Section 8 and must agree to maintain highly effective contraception as defined in Section 8 during the study.
15. Any medical condition, which in the opinion of the Investigator, may place the patient at higher risk from his/her participation in the study, or is likely to prevent the patient from complying with the requirements of the study or completing the study.

6.3 Co-enrolment Guidelines

To avoid potentially confounding issues, ideally participants should not be recruited into other trials. All potential participants must be asked whether they are involved in any other research study prior to randomisation into TEMPEST. If the individual is involved in any other research study, the study team must investigate whether the other study allows for co-enrolment, and whether the individual is eligible for TEMPEST. Individuals who are participating in a trial testing a medicinal product at the time of enrolment, or within 30 days or 5 half-lives of enrolment, will be ineligible for the TEMPEST trial. Where recruitment into another trial is considered to be appropriate and without having any detrimental effect on the TEMPEST trial or any other trial, this must first be discussed with the coordinating centre (LCTC) who will contact the CI, Dr Chris Miller.

7 RECRUITMENT AND RANDOMISATION

7.1 Participant Identification and Screening

Potential participants will be identified from locations such as clinics, diagnostic departments, wards, patient registries, medical records and databases.

Potential participants who may be eligible to take part in the trial will initially be approached by health care professionals involved with their clinical care. The clinical team will discuss the trial and issue the PISC to the patient/organise for the PISC to be issued to the patient. If appropriate, and if the clinical team have not given the invitation letter and PISC form to potential participants already, the invitation letter and PISC form will be sent (by post, email or other means) to potential participants. Potential participants may be contacted, for example by phone or email, at least seven days from when the PISC is given/sent to them to ensure that they have received it, review all inclusion and exclusion criteria that can be determined at that point and invite them to Visit 1.

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

If a patient is not eligible, the study team may consider reassessment of the patient at a later time if the study team believe that the patient's condition has changed and may potentially be eligible.

7.2 Informed Consent (Visit 1)

Informed consent is required for all participants in this trial. Informed consent is a process initiated prior to an individual agreeing to participate in a trial and continues throughout the individual's participation.

Consent from the patient should be obtained prior to participation in the trial, after a full explanation has been given of the treatment options, including the conventional and generally accepted methods of treatment.

Written versions of the PISC forms will be presented to the patients detailing no less than: the exact nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part.

Discussion of objectives, risks and inconveniences of the trial and the conditions under which it is to be conducted are to be provided to patients by staff with experience in obtaining informed consent. The PISC will detail the trial interventions/products, trial procedures and risks and will be approved by a REC. The patient will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the patient. All patients will be given opportunity to ask any questions that may arise, should have the opportunity to discuss the study with their surrogates and time to consider the information prior to agreeing to participate. A contact point where further information about the trial may be obtained will be provided.

It will be clearly stated that participation in the trial is voluntary and that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal. The right of the patient to refuse consent to participate in the trial without giving reasons must be respected.

In obtaining and documenting informed consent, the investigator should comply with applicable regulatory requirements and should adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki.

The participant may, without being subject to any resulting detriment, withdraw from the trial at any time by revoking the informed consent. The rights and welfare of patients will be protected by emphasising to them that the quality of medical care will not be adversely affected if they decline to participate in this study.

The patient and the person taking consent must personally sign and date the latest approved version of the informed consent form, during Visit 1 and before any study specific procedures are performed. Procedures may be performed in advance of written informed consent being obtained if they are part of the usual standard of care. These may include, for example, routine laboratory tests and cardiac imaging. Data cannot be transferred to the LCTC until consent has been obtained.

The patient will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator or an independent party to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the informed consent. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Principal Investigator. A copy of the signed Informed Consent will be given to the participants. The original signed form will be filed in the Investigator Site File (ISF) and a copy will be filed in the participant's notes retained at the study site. One final copy of the consent form should be sent to the LCTC via secure email or post within 7 days of completion and separately to pseudonymised trial documents.

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

7.3 Baseline (Visit 1)

The following procedures will be performed at Visit 1 (see Table 1 in Section 8 for further details):

1. Informed consent. Only once written informed consent has been provided (see section 7.2) can the assessments listed below be performed.
2. Review of demographic and baseline characteristics.

   Data to be collected include: date of birth, sex and ethnicity.
3. Review of medical history.

   Relevant medical history/current medical condition data, including HCM genotype if available, will be recorded.
4. Assessment of eligibility criteria.
5. Review of concomitant medications.

   All relevant medications will be recorded. Relevant medications are defined as:

   - Anticoagulant medications
   - Antiplatelet medications
   - Angiotensin-converting enzyme inhibitors
   - Angiotensin II receptor blockers
   - Angiotensin receptor-neprilysin inhibitors
   - Antiarrhythmic medications
   - Beta-blockers
   - Calcium channel blockers
   - Cholesterol-lowering medications
   - Digoxin
   - Diuretics
   - Nitrates
6. Pulse rate, blood pressure, height and weight will be recorded.

7. Laboratory investigations.

   A blood sample (approximately 20-25ml in total) will be drawn for laboratory tests that will include: blood count, renal function, pregnancy test (as appropriate), iron, copper, caeruloplasmin, high sensitivity troponin.

8. A urine sample will be provided to participants with instructions to give an early morning sample on the day of Visit 2 and to bring to Visit 2

9. ECG.

   May be performed at Visit 1 or Visit 2

10. 24-hour heart monitor.

7.4 Confirmation of eligibility, special cardiac procedures and randomisation (Visit 2)

If the assessments performed at Visit 1 show that the participant is eligible for the study, Visit 2 will be arranged. Visit 2 should take place within 28 days (4 weeks) of Visit 1 i.e. if Visit 1 takes place on the 1st of the month, Visit 2 should take place on or before the 29th of the month. The following procedures will be performed at Visit 2 (see Table 1 in Section 8 for further details)

1. Female participants of childbearing capacity will be asked if there is a chance that they could have become pregnant since Visit 1. If the participant confirms that there is a chance, then a urine pregnancy test will be performed.

2. Confirmation of eligibility.

   Full eligibility may only be confirmed by a doctor who has been authorised to do so on the site Delegation Log; a record of this confirmation must be made in the patient's medical notes. Site research teams may contact the CI via the LCTC for queries regarding eligibility.

   The following procedures should only be performed when eligibility is confirmed.

3. Collection of urine sample from participant

4. CMR scan. A blood sample (approximately 5ml) will be drawn for measurement of haematocrit as part of the CMR scan procedure.

5. CPET

6. ^{31}P MRS in a subgroup of participants.

7. Randomisation

A participant may only be randomised once full eligibility has been confirmed by a doctor and written informed consent has been obtained.

Patients will be randomised in a 1:1 ratio to receive either trientine or placebo.

Randomisation will be accomplished over the internet using web randomisation software accessed using a secure website provided via the LCTC. Block randomisation, stratified by site, will be implemented, with computer generated randomisation allocations and randomly varying block sizes.

At Visit 2, an authorised member of the research team will access the web randomisation software. After entering the participant's study number, and confirming that the participant qualifies for randomisation, the randomisation software will assign a randomisation number to the patient. The randomisation software will be used to link the patient to a treatment arm. The randomisation software will generate an email that will be sent to the researcher and a copy of the email will also be sent to the site PI, the site clinical trials pharmacy team and the LCTC TEMPEST trial team.

Following this, an authorised, clinically trained member of the research team will access the IMP management software. After entering the participant's randomisation number and details of the visit, the software will allocate

an appropriate number of bottles to the participant. Then an authorised, clinically trained member of the research team can complete the prescription form and this will be given to the pharmacy team.

The same process will occur at the subsequent dispensing visits (Visits 3, 4 and 5) i.e. an authorised member of the research team will access the IMP management software to allocate an appropriate number of bottles to the participant and complete the prescription form with the specified number of bottles. The software will generate an email that will be sent to the site pharmacy.

Randomisation numbers will be generated to ensure that treatment assignment is unbiased and concealed from patients and investigator staff. A patient randomisation list will be produced by the web randomisation provider using a validated system that automates the random assignment of patient numbers to randomisation numbers. These randomisation numbers are linked to the different treatment arms.

Randomisation: web access
https://ctrc.liv.ac.uk/Randomisation/TEMPEST

If there are any problems with the randomisation systems contact the coordinating CTU on 0151 794 9763 or via email on tempest@liverpool.ac.uk

(Note that LCTC is open from 0900 – 1700, Monday – Friday, excluding public holidays and University closure days)

Participant diaries and instructions will be given to participants at Visit 2.

Central back up randomisation process:
In the event of a randomisation system failure, the centre should contact the coordinating team in LCTC (Monday to Friday between 9:00 to 17:00 excluding bank holidays and University closure days) to try to resolve the problem. If the problem cannot be resolved the LCTC will perform central randomisation and randomise the participant using the back-up randomisation system. The back-up randomisation system is an exact replica of the live system but is based on a standalone PC at LCTC.

7.5 Who is Blinded to Allocations

This will be a double-blind study. The placebo and active treatments will appear identical and will be dispensed in identical containers with identical labelling. All trial participants, care providers, outcome assessors and data analysts will remain blinded throughout the study. Pharmacy staff at each participating hospital will remain unblinded. For unblinding procedures see section 9.3.

8 PARTICIPANT TIME LINE, ASSESSMENTS AND PROCEDURES

8.1 Schedule for Follow-up

All participants, including those who discontinue the study medication before completing the study, should continue attending the scheduled visits as outlined in Table 1 until the study ends.
If one visit is postponed or brought forward, it should not result in the next visit being postponed or brought forward. The next visit, if at all possible, should adhere to the original time schedule.
All follow up visits will be scheduled from the date of randomisation. Each participant will be followed up for 52 weeks after randomisation. During this time participants will attend scheduled follow up visits.

8.1.1 Follow-up visits

Each participant will have 4 follow-up visits and these will be conducted at 13, 26, 39 and 52 weeks. Please refer to Table 1 for the acceptable time windows for each visit.

8.1.2 Unscheduled visits

At the investigator's discretion, a participant may attend for an unscheduled study visit. For example, if a participant contacts the research team describing an adverse event, the investigator may deem it necessary to assess the participant in clinic. Please refer to Table 1 for further details.

8.1.3 Long Term Follow-Up

Long term follow-up will be performed using routinely collected data (e.g. data held by NIHS Digital) with appropriate linkage. This data will not be collected as part of the main study. Participants will be asked to consent to their medical data held by NHS Digital being accessed and that they agree to allow information or results arising from this study to be used in future healthcare and/or medical research providing their confidentiality is maintained, in order to facilitate this. This research will be performed by the CI and his research team, at Manchester University NHS Foundation Trust (i.e. the Sponsor).

Table 1. Visit Schedule

Visit		**1**	**2**	**3**	**4**	**5**	**6**	
Time and acceptable window (weeks)		-4	0*	13 ± 1	26 ± 2	39 ± 2	52 ± 2	
Procedures		Screening and consent	Randomisation				End of treatment, end of trial	Unscheduled visit
Signed Consent Form		X						
Demographics		X						
Review of Medical History		X						
Assessment of Eligibility Criteria		X	X					
Review of Concomitant Medications		X	X	X	X	X	X	X
Pulse, blood pressure		X		X	X	X	X	X^
Height, weight		X					X	X^
Give urine sample bottle to participant		X	X	X	X	X		X^
Clinical laboratory	Urine test**		X	X	X	X	X	
	Pregnancy Test***	X	X[#]	X	X	X	X	X^
	Blood tests (safety)****	X		X	X	X	X	X^
	High sensitivity troponin blood test	X					X	
	Haematocrit blood test*****		X					
	Sample handling/processing for central analysis	X	X	X	X	X	X	X^
	Specimen dispatch by post/courier	X	X	X	X	X	X	X^
Special procedure	ECG	X[§]		X	X	X	X	X^
	CMR scan		X				X	
	24hr heart monitor	X					X	

	Cardiopulmonary exercise test		X				X	
	31 Phosphorus spectroscopy (subgroup)		X				X	
Randomisation			X					
Prescription of IMP			X	X	X	X		
Pharmacy dispensing of IMP			X	X	X	X		
Dispense participant diary and instruct			X					
Pharmacy collection and recording of unused medication to assess compliance				X	X	X	X	X^
Review patient diary				X	X	X	X	X^
Review/reporting of AEs/SAEs				X	X	X	X	X
eCRF completion including data transfer and query resolution		X	X	X	X	X	X	X

*Visit 2 (Randomisation) should take place within 28 days (4 weeks) of Visit 1. See Section 7.4

**Urine test (efficacy): Early morning urine collection for urine copper

***Applies to females of childbearing capacity only. A serum pregnancy test will be performed at Visit 1. Urine pregnancy tests will be performed at subsequent visits.
At Visit 2, female participants of childbearing capacity will be asked if there is a chance that they could have become pregnant since Visit 1. If the participant confirms that there is a chance, then a urine pregnancy test will be performed.

****Blood tests (safety): blood count, renal function, serum iron, serum copper and serum caeruloplasmin.

*****Haematocrit blood test (used as part of the CMR measurements)

§May be performed at Visit 1 or Visit 2 but must be done before randomisation

^Optional procedures performed at the investigator's discretion

8.2 Study Procedures

Study procedures will be performed as detailed in the Study Reference Manual, at the time points specified in Table 1. Briefly, procedures will include:

8.3 Procedures for assessing efficacy and mechanism

8.3.1 Cardiovascular magnetic resonance (CMR)

CMR is the gold standard for measurement of LVMi, LV volumes, LV ejection fraction, left atrial volume and left atrial ejection fraction.[28] CMR provides robust, highly reproducible measurement of myocardial fibrosis.[29-34] CMR provides accurate and reproducible measurement of LV and left atrial strain.[35-37]

CMR scanning will be performed according to standard protocols including administration of gadolinium-based contrast agent and same-day measurement of haematocrit.[38] CMR will be performed at 3T where possible and the same scanner should ideally be used for baseline and follow-up scans. The blood sample for haematocrit at Visit 2 will be drawn as part of the CMR scan procedure and analysed at local laboratories at sites. The haematocrit at Visit 6 will be available from the blood count performed as part of the safety blood tests. Participants with an estimated glomerular filtration rate (eGFR) < 30 millilitres/minute will not receive gadolinium-based contrast agent. Sequences will include cine imaging and tissue characterisation.

CMR scan measurements will be performed centrally. Measurements will include indices of ventricular mass, volumes and function (including strain), atrial volumes and function (including strain) and myocardial composition including myocardial extracellular and cellular indices.

8.3.2 24-hour heart monitor

24-hour ambulatory monitoring is a standard clinical method for evaluating heart rhythm. Recorded measurements will include the frequency and burden of abnormal heart rhythms. Analysis will be performed centrally.

8.3.3 Cardiopulmonary exercise test (CPET)

CPET is the gold standard for assessment of exercise capacity. CPET will be performed according to published recommendations.[39] Recorded measurements will include indices of exercise time, exercise tolerance and functional capacity. Analysis will be performed centrally.

8.3.4 ^{31}Phosphorus magnetic resonance spectroscopy (^{31}P MRS)

^{31}P MRS provides a non-invasive measurement of myocardial energy metabolism. ^{31}P MRS will be performed in a subgroup of patients. A standard acquisition technique will be used, as descried previously.[22, 40] MRS measurements will be performed centrally and will include PCr/ATP ratio.

8.3.5 Clinical laboratory tests

High sensitivity troponin provides assessment of cardiomyocyte injury. Blood sampling for high sensitivity troponin will be performed at the time points as set out in Table 1. The total volume of blood collected at each visit will be approximately 20-25ml, of which approximately 5ml will be used for the high sensitivity troponin analysis. Samples for high sensitivity troponin analysis will be sent to a central laboratory for analysis. Results will not routinely be reported to investigators/sites. Further details on the collection, shipment of samples and reporting of results will be provided in the Study Reference Manual.

Urinary copper excretion will be assessed using first morning urine samples. Urine sampling will be performed at the time points set out in Table 1. Sample pots should be given to participants on the preceding visit in order to allow sampling to take place. The volume of urine required at each visit will be up to approximately 20ml.

Urine samples will be sent to a central laboratory for analysis. Results will not routinely be reported to investigators/sites. Further details on the collection, shipment of samples and reporting of results will be provided in the Study Reference Manual.

8.4 Procedures for Assessing Safety

8.4.1 Electrocardiogram (ECG)

Standard 12-lead ECG.

8.4.2 Clinical laboratory tests

Blood sampling for blood count, renal function, serum iron, serum copper and serum caeruloplasmin will be performed at the time points as set out in Table 1. The total volume of blood collected at each visit will be approximately 20-25ml.

Approximately 10-15ml of the blood sample will be used for measurement of blood count, renal function and serum iron. This analysis will be performed at local laboratories at sites. Further details on the collection and reporting of results will be provided in the Study Reference Manual.

Approximately 5-10ml of the blood sample will be used for measurement of serum copper and serum caeruloplasmin. Samples for serum copper and serum caeruloplasmin analysis will be sent to a central laboratory for analysis. Results will be reported to investigators/sites. Further details on the collection, shipment of samples and reporting of results will be provided in the Study Reference Manual.

8.4.3 Pregnancy assessments

Female participants of childbearing capacity will have a serum pregnancy test performed at Visit 1 and urine pregnancy tests at Visits 3, 4, 5, 6. At Visit 2, female participants of childbearing capacity will be asked if there is a chance that they could have become pregnant since Visit 1. If the participant confirms that there is a chance, then a urine pregnancy test will be performed.

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

- Combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation
    - Oral
    - Intravaginal
    - Transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation
    - Oral
    - Injectable
    - Implantable
- Intrauterine device (IUD)
- Intrauterine hormone-releasing system (IUS)
- Bilateral tubal occlusion
- Vasectomised partner

- True sexual abstinence (when this is in line with the preferred and usual lifestyle of the participant). Periodic abstinence is not an acceptable form of highly effective contraception.

8.4.4 Assessment for adverse events

An assessment of adverse events will be undertaken by a delegated member of the research team. Requirements for adverse event reporting is detailed in Section 10.

8.5 Other procedures

8.5.1 Participant diary

One participant diary will be provided to the participant for the duration of the trial. This will include sections for recording visit appointment dates and times, side effects/adverse events and missed IMP doses. If the diary is misplaced or becomes full, another diary will be provided to the participant. Data from the participant diary will not be entered onto the trial database.

8.6 Patient Transfer and Withdrawal

In consenting to the trial, participants are consented to trial treatment, follow-up and data collection. If voluntary withdrawal occurs, the participant should be asked to allow continuation of scheduled evaluations, complete an end-of-study evaluation if appropriate, and be asked to allow continuation of data collection and use of that data. The participant should also be given appropriate care under medical supervision until the symptoms of any adverse event resolve or the participant's condition becomes stable.

Follow-up of participants will be continued through the trial unless the participant explicitly also withdraws consent for follow-up.

8.6.1 Patient Transfers

For patients moving from the area, every effort should be made for the patient to be followed-up at another participating trial centre and for this trial centre to take over responsibility for the patient or for follow-up via GP. A copy of the patient Case Report Forms (CRF) should be provided to the new site. The patient remains the responsibility of the original site until the new site PI has signed the Transfer CRF.

8.6.2 Premature discontinuation of trial treatment

Participants may be withdrawn from treatment for any of the following reasons:

1. Withdrawal of consent by participant.
2. Unacceptable toxicity (may include serious adverse events [SAEs] related to study treatment).
3. Pregnancy.
4. Intercurrent illness preventing continuation.
5. A change in the participant's condition that justifies the discontinuation of treatment in the opinion of the clinical team or principle investigator.

Participants who discontinue study treatment early must be asked to return all unused study treatment and any empty medication bottles.

Early discontinuation of study treatment should be reported to the LCTC as soon as possible. Dose adjustments (see section 9.2.3) should also be reported to the LCTC as soon as possible.

If a participant wishes to discontinue trial treatment, the research team should nevertheless explain the importance of remaining on trial follow-up, or failing this, of allowing routine follow-up data to be used for trial

purposes. Generally, follow-up and data collection will continue unless the participant explicitly also withdraws consent for follow-up (see section 8.6.3).

Participants that discontinue trial treatment before visit 3 will not be expected to complete visits 4 and 5 and as such, incompletion of visits 4 and 5 for participants that have discontinued trial treatment before visit 3 will not be classed as a protocol deviation. Participants who discontinue study treatment should complete all visit 6 (End of Study) assessments and procedures.

8.6.3 Withdrawal from Trial Follow-up

Participants are free to withdraw consent at any time without providing a reason. The clinician may also withdraw the participant if required, this will include situations such as death of the participant or if the participant is transferred to another site and is unable to complete follow-up. Participants who wish to withdraw consent for the trial will have pseudonymised data collected up to the point of withdrawal of consent included in the analyses. The participant will not contribute further data to the study and the LCTC should be informed in writing and a withdrawal CRF should be completed.

8.6.4 Withdrawal from Trial Completely

The participant's rights to access, change or move their information is limited, as the information needs to be managed in specific ways in order for the research to be reliable and accurate. If the participant withdraws from the study, the information that has already been obtained about them will be kept. To safeguard the participant's rights, the minimum personally-identifiable information will be used as possible.

It is critical to the integrity of this study that patients adhere to the visit schedule outlined in the protocol. As such, every reasonable effort should be made to convey the importance of remaining on the study to participants. Any participant withdrawing from the trial completely must be reported to the LCTC to discuss the circumstances of the case in an effort to ensure patient safety and appropriate documentation of events.

8.7 Subgroup

8.7.1 Rationale

Energy depletion is widely hypothesised to be an important HCM disease mechanism. Trientine is associated with restoration of mitochondrial structure and function and energy metabolism. It is therefore hypothesised that the reduction in LV mass with trientine will be mediated by improved myocardial energetics, which will be determined by increased copper excretion. See Section 3 for further details.

8.7.2 Objectives

See Section 3.4.2. Mechanistic objectives. In brief, alongside the other mechanistic evaluations, the subgroup will determine whether:

- Trientine, compared to placebo, leads to an improvement in myocardial energetics.
- LV hypertrophy regression is mediated by cellular regression, extracellular regression or improved myocardial energetics, which are in turn determined by copper excretion.

8.7.3 Design

A subgroup of 84 patients (aiming for 42 in each treatment group) will undergo ^{31}P MRS as part of the mechanistic evaluation. ^{31}P MRS provides a non-invasive measurement of myocardial energy metabolism.

Participant selection will be consecutive until the required number are recruited.

8.7.4 Assessment and procedures

^{31}P MRS is described in section 8.2. It will be performed at the time points specified in Section 8.1.

8.8 Loss to Follow-up

Contact details will be confirmed with participants at the beginning of the study. Participants will generally be contacted by phone. If participants miss a visit a member of the research team will contact them to determine the reason and rearrange the visit.

8.9 Trial Closure

The end of the trial is defined as the date on which data for all participants is frozen and data entry privileges are withdrawn from the trial database. However, the trial may be closed prematurely by the TSC, on the recommendation of the IDSMC.

9 TRIAL TREATMENT/INTERVENTIONS

9.1 Introduction

Participants will be randomised to receive trientine or placebo for 52 weeks.

Rationale for trientine: See Introduction.

Dose rationale: Trientine dihydrochloride 1200mg/day is chosen because it was the dose used in the aforementioned studies in diabetes and HCM, in which it was associated with the described beneficial/potentially beneficial effects, and was safe and well tolerated.[16, 18]

Duration rationale: A duration of 52 weeks is chosen because it is felt to be the shortest period within which efficacy can be demonstrated. In the aforementioned pilot study in HCM, 6 months of trientine was associated with trends towards reductions in LVMi and fibrosis.[18] In the trial of trientine in diabetes, 12 months of trientine was associated with a significant reduction in LVMi, and, importantly, the reduction in LVMi at 12 months was double that at 6 months.[16] It is therefore felt that 12 months is the optimal duration to balance sufficient time to demonstrate efficacy, with value for money and minimising patient burden.

9.2 Investigational Medicinal Product (IMP)

Active intervention: trientine dihydrochloride 1200 mg per day. This shall be taken orally as two Cufence 200 mg hard capsules two times per day.

(Each Cufence 200 mg hard capsule contains 200 mg of trientine which is equivalent to 300 mg trientine dihydrochloride. Therefore Cufence 800 mg per day is equal to trientine 800 mg per day and trientine dihydrochloride 1200 mg per day. In order to minimise the potential risk of confusion regarding doses, and because Cufence is written on the capsules, the name Cufence is used for the active intervention).

Control intervention: placebo (manufactured to appear identical to Cufence 200 mg hard capsules), taken as two capsules two times per day.

9.2.1 Formulation, Packaging, Labelling, Storage and Stability

9.2.1.1 Formulation and packaging

The IMP will be supplied by Univar Solutions BV to the pharmacy departments at each site.

IMP will be supplied in amber glass bottles with a polypropylene cap and induction heat seal liner with a sachet of dried silica gel as desiccant. There will be 100 capsules per bottle, which will be labelled for investigational use only. IMP will be supplied as hard white, oval-shaped capsules printed with ‘Cufence’ in grey ink that are visually indistinguishable.

The clinical formulation of Cufence is detailed in the SmPC. Each final product batch will be supplied to the sites with Qualified Person (QP) release certificate documentation.

9.2.1.2 Labelling

Univar Solutions BV will supply the bottles with the main label. Packaging and label will be compliant with Good Manufacturing practice (GMP) and Good Clinical practice (GCP) and Annex 13.

9.2.1.3 Storage and shelf life

IMP will be stored in pharmacy departments at each site.

Storage requirements are detailed in the SmPC. Unopened bottles should be stored below 25°C. No other special storage is required.

After bottles are opened, they should be stored in a refrigerator (at approximately 2ºC-8ºC). Bottles should be kept tightly closed in order to protect from moisture.

The shelf life of unopened bottles is 3 years. After first opening, bottles have a shelf life of 3 months.

9.2.1.4 Disposal

Disposal will take place after full reconciliation of batches and will be the responsibility of the pharmacy departments at each site under the guidance of the Sponsor. Sites will only do this after receiving written approval from the Sponsor. Proof of disposal will be provided by each site to Sponsor, who will inform Univar Solutions BV.

9.2.2 Preparation, Dosage and Administration of Study Treatment/s

9.2.2.1 Dispensing

The Pharmacy departments at each site will dispense the IMP to participants. The IMP will be dispensed to participants every 13 weeks, but may be dispensed at other visits, as needed. Participants will be instructed to store study treatments in the fridge. Participants will be instructed to use study treatments in the order in which they are dispensed, to open and use one bottle at a time and that they must return all used and unused study treatment bottles.

9.2.2.2 Dosing regimen

Two capsules two times per day.

Participants will remain on this dose for the duration of the study period (52 weeks) unless the dose is reduced to manage an adverse event. Doses above two capsules two times per day are not recommended for any participant.

9.2.2.3 Method of administration

Capsules will be taken orally. Each dose should be taken with water at least 1 hour before or 2 hours after food, and at least one hour apart from any other medicinal product, food, or milk, at the same times each day. Capsules should be swallowed whole and should not be opened. Capsules should not be used if they become sticky or wet.

9.2.2.4 Missed doses

If a participant misses a scheduled dose, that dose should be skipped. Regular dosing should resume with the next scheduled dose. Participants should not take any extra doses to make up for missed doses.

9.2.3 Dose Modifications

IMP dose may be reduced in participants experiencing adverse events.

In participants who experience nausea or skin rash, or other mild symptoms, the IMP dose may be reduced to 1 capsule twice per day, or, if the symptoms persist, 1 capsule once per day. If symptoms continue, participants may be instructed to interrupt treatment for 1 to 2 weeks to allow symptoms to resolve. Re-escalation to the standard maintenance dose (2 capsules two times per day) may be performed as tolerated.

In participants who experience a significant fall in haemoglobin or serum iron, defined as haemoglobin or serum iron falling below the local site normal reference range, the test should be repeated and if confirmed, the IMP dose may be reduced to 1 capsule twice per day or 1 capsule once per day as felt to be clinically appropriate and haemoglobin and iron should be monitored. Iron supplementation may be given. If the haemoglobin or serum iron do not improve above this level, participants may be instructed to interrupt treatment for 1 to 2 weeks to allow it to resolve. Re-escalation to the standard maintenance dose (2 capsules two times per day) may be performed as felt to be clinically appropriate.

In participants who experience a significant fall in serum copper, defined as copper falling below the central normal reference range, the test should be repeated and if confirmed, the IMP dose may be reduced to 1 capsule twice per day or 1 capsule once per day as felt to be clinically appropriate and serum copper and caeruloplasmin should be monitored. If serum copper does not improve above this level, participants may be instructed to

interrupt treatment for 1 to 2 weeks to allow it to resolve. Re-escalation to the standard maintenance dose (2 capsules two times per day) may be performed as felt to be clinically appropriate.

If participants experience severe diarrhoea or abdominal pain IMP should be stopped immediately. IMP should only be restarted after discussion with the CI or designated representative co-ordinated by the LCTC.

Dose adjustments will be reported to the LCTC via the eCRFs.

9.2.4 Overdose of the IMP

Experience with doses higher than the recommended therapeutic dose is limited. In the event of overdose the patient should be observed, appropriate biochemical analysis performed and symptomatic treatment given. There is no antidote.

Occasional cases of trientine overdose have been reported. In cases up to 20 g of trientine base there were no apparent adverse effects reported. A large overdose of 40 g of trientine base resulted in self-limiting dizziness and vomiting with no other clinical sequelae or significant biochemical abnormalities reported.

9.2.5 IMP contraindications

Hypersensitivity to the active substance or to any of the excipients listed in the SmPC, which include:

1. Capsule content: Magnesium stearate, Colloidal anhydrous silica.

2. Capsule shell: Gelatin, Titanium dioxide.

3. Printing ink: Shellac, Propylene glycol, Titanium dioxide, Iron oxide black, Iron oxide yellow.

9.2.6 Accountability Procedures for Study Treatment/s

The study medication will be supplied by Univar Solutions BV to the Pharmacy departments at each site. All movements of study medication between Univar Solutions BV and the sites will be documented.

The investigator will transcribe the number of bottles allocated to the participant at that visit by the IMP management system onto the prescription form. The IMP management system will generate an email to the site pharmacy team stating the number of bottles allocated to that participant at that visit.

Participants will be asked to bring all unused medication to all visits. All unused medication will be returned to the pharmacy department at each site at each dispensing visit (i.e. visits 3, 4, 5) and at the end of study visit (visit 6).

Accountability logs will be completed by the Pharmacy departments at each site. This will include the number of capsules returned by each participant. A copy of the log will be provided to the LCTC at the end of each month.

9.2.7 Assessment of Compliance with Study Treatment/s

Participants will be asked to bring all unused medication to all visits. All unused medication will be returned to the pharmacy department at each site at each dispensing (i.e. visits 3, 4, 5) and at the end of study visit (visit 6).

Individual participant compliance will be assessed by the number of unused capsules returned by each participant, which will be recorded on the accountability logs completed by the Pharmacy departments at each site. This will be recorded at each dispensing visit (i.e. visits 3, 4, 5) and at the end of study visit (i.e. visit 6), and any unscheduled visits where appropriate. Double-blind study drug exposure will be calculated based upon the returned capsules recorded in the Pharmacy accountability logs.

The study team will review participants' diaries and discuss compliance during study visits. The study team will counsel participants if they are noncompliant. Diaries will not be used to formally record compliance.

9.3 Unblinding

9.3.1 Before unblinding treatment allocation

Unblinding of participants during the conduct of the clinical trial will not be allowed unless there are compelling medical or safety reasons to do so. Unblinding must be essential to either enable treatment of serious adverse event/s, or to enable administration of another therapy that is contraindicated by the trial treatment.

In order to fulfil delegated activities associated with pharmacovigilance, LCTC may be required to unblind an individual participant for the purposes of safety reporting. Upon receipt of a complete Serious Adverse Event (SAE) report, designated staff at the LCTC (usually the trial coordinator named in the protocol) will liaise with the CI or their designated other, who will evaluate seriousness, expectedness and causality. Cases that are considered serious, unexpected and possibly, probably or almost certainly related to the treatment under investigation (i.e. possible SUSARs) will be unblinded at the LCTC by a delegated individual and, where applicable, reported to regulatory authorities and the ethics committee within the required timescale.

9.3.2 In-hours unblinding request (non-emergency)

For non-emergency unblinding requests, the pharmacist or delegate will ring LCTC and provide the details of the unblinding request to the LCTC Trial Coordinator (ensuring no patient identifiable information is passed on). The LCTC Trial Coordinator will then liaise with the CI/designated other to confirm whether unblinding is necessary. If the CI/designated other confirm that unblinding is deemed necessary, the LCTC Trial Coordinator will contact pharmacy and ask that the treatment allocation is revealed to the individual who issued the unblinding request.

The delegated individual at pharmacy will complete an unblinding CRF and a copy must be forwarded to the LCTC within 24 hours.

9.3.3 Out-of-hours unblinding request (non-emergency)

For non-emergency unblinding requests, the pharmacy team will contact the CI, or designated other, directly to confirm if unblinding is necessary via the 24-hour contact number. Alternatively, if there is an issue with this contact number, the pharmacy team will contact the CI, or designated other, via switchboard at Manchester University NHS Foundation Trust. If the CI, or designated other, confirm that unblinding is necessary, pharmacy will contact the person who issued the unblinding request to reveal treatment allocation.

In all cases, the pharmacy team will inform the CI and the LCTC when the unblinding has occurred and give the identity of all recipients of the unblinding information. The pharmacy team will complete an unblinding CRF and a copy must be forwarded to LCTC within 24 hours.

The allocation should not be revealed to the CI. The allocation should not routinely be revealed to LCTC personnel (treatment allocation is not recorded on the unblinding CRF) unless this is required to meet pharmacovigilance responsibilities.

9.3.4 Emergency unblinding

In the event of an emergency, the decision to unblind a participant resides with the Investigator at the recruiting site. In this situation, the pharmacist or delegate should reveal the treatment allocation to the Investigator without approval from the CI or LCTC. Investigators carrying out emergency unblinding must be satisfied that it is a genuine emergency and that knowledge of the treatment allocation is needed to guide the appropriate clinical management of the participant. It is recommended that where possible research staff at site contact the LCTC in the first instance if there is any uncertainty about what constitutes an emergency and whether unblinding can be avoided e.g. if treatment can simply be stopped.

The reason for unblinding must be recorded on the unblinding CRF which should be provided to the LCTC as soon as possible.

All non-emergency unblinding requests must be made following sections 9.3.2 and 9.3.3.

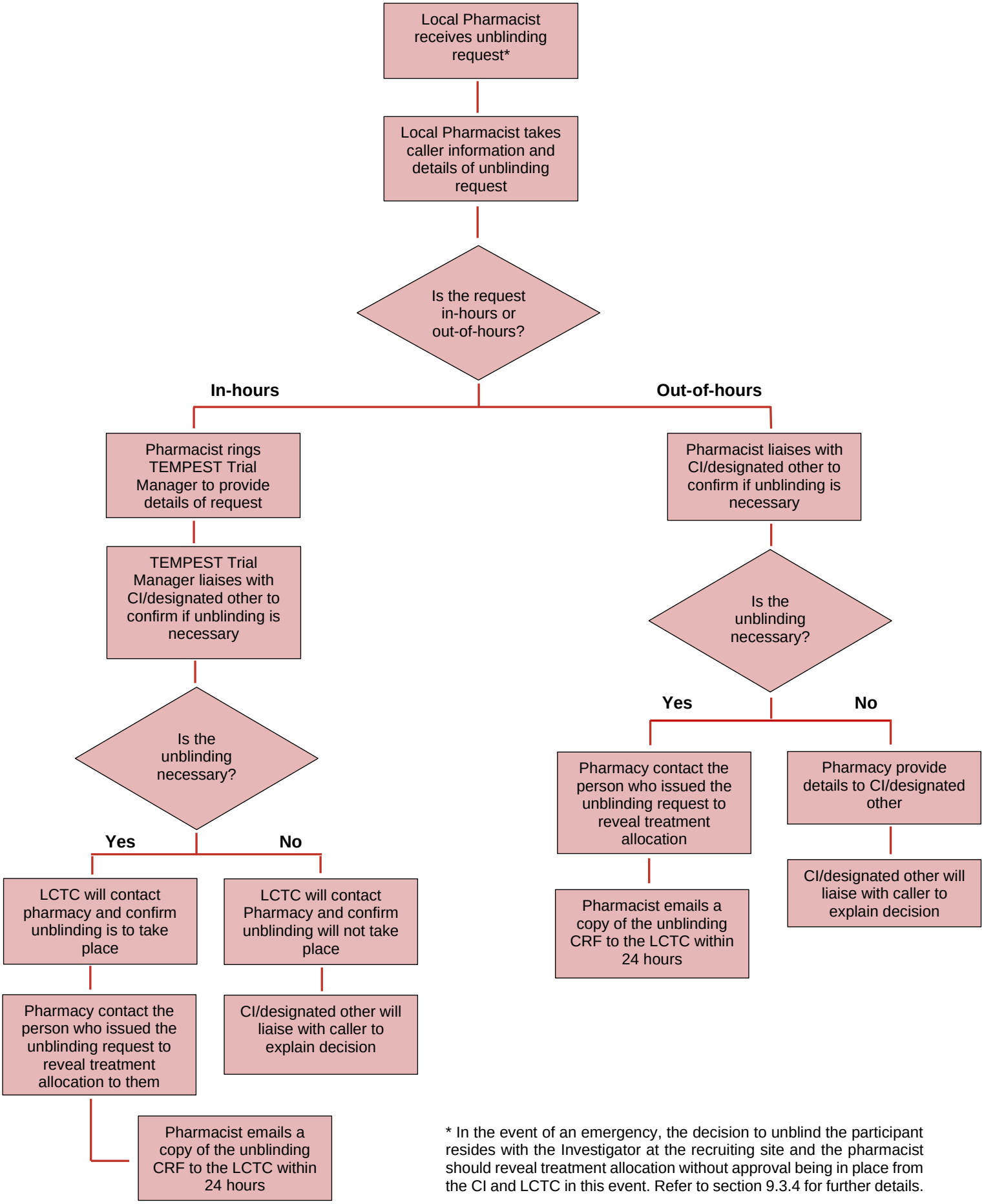
9.3.5 Accidental unblinding

For accidental unblinding within the LCTC, an Accidental Unblinding Report should be sent directly to LCTC Operational Team. The completed report should also be reviewed by the TMG. All instances of inadvertent unblinding outside of the LCTC should be recorded in writing and reported to the LCTC using the same template. The Trial Coordinator will provide the template to site when necessary. The completed report should be reviewed within the TMG who will determine whether the preventative action is deemed appropriate and the Trial Coordinator will feedback to sites as to whether any further action is required.

9.3.6 End of trial

In line with the principles of GCP, any intentional or unintentional breaking of the blind will be reported and explained in the clinical trial report at the end of the trial.

Unblinding Guidance Diagram (non-emergency):



* In the event of an emergency, the decision to unblind the participant resides with the Investigator at the recruiting site and the pharmacist should reveal treatment allocation without approval being in place from the CI and LCTC in this event. Refer to section 9.3.4 for further details.

9.4 Concomitant Medications/Treatments

Please refer to the SmPC regarding interaction with other medicinal products and other forms of interaction.

9.4.1 Medications Permitted

Participants are permitted to receive licensed treatments for cardiovascular disease and other conditions.

9.4.2 Medications Not Permitted/ Precautions Required

1. Concomitant oral iron should be administered at a different time than IMP.
2. There is no evidence that calcium and magnesium antacids alter the efficacy of trientine but it is recommended to separate the timing of their administration from when IMP is administered.
3. The SmPC states that combination of IMP with zinc is not recommended (for the treatment of Wilson disease) as it may reduce effect. Zinc is not used as a treatment for any form of cardiovascular disease and patients with HCM would not be expected to be taking zinc, nevertheless, participants will be advised to avoid zinc tablets in the participant information sheet.
4. The SmPC states that “Lupus-like reactions (symptoms may include persistent rash, fever, joint pain, and tiredness) have been reported in some patients switched to trientine medicine after D-penicillamine medicine. However, it was not possible to determine if the reaction was due to trientine or to previous D-penicillamine treatment”. D-penicillamine is not used as a treatment for any form of cardiovascular disease and patients with HCM would not be expected to be taking D-penicillamine.

9.4.3 Data on Concomitant Medication

All relevant concomitant medications will be recorded at baseline and change in concomitant medication will be recorded at each visit in the eCRFs. Medication name will be recorded.

Relevant medications are defined as:

- Anticoagulant medications
- Antiplatelet medications
- Angiotensin-converting enzyme inhibitors
- Angiotensin II receptor blockers
- Angiotensin receptor-neprilysin inhibitors
- Antiarrhythmic medications
- Beta-blockers
- Calcium channel blockers
- Cholesterol-lowering medications
- Digoxin
- Diuretics
- Nitrates

10 SAFETY REPORTING

10.1 Time Period for Safety Reporting

Safety reporting of Serious Adverse Events or Reactions will be reported during the clinical trial from the period of informed consent until visit 6.

10.2 Terms and Definitions

The definitions below are based on The Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031) definitions of Adverse Events:

Adverse Event (AE)

Any untoward medical occurrence in a participant to whom an IMP has been administered, including occurrences which are not necessarily caused by or related to that product.

Adverse Reaction (AR)

Any untoward and unintended response in a participant to an IMP which is related to any dose administered to that participant.

N.B. All cases judged by the reporting investigator as having a reasonable suspected causal relationship to the IMP, i.e. the relationship cannot be ruled out, qualify as Adverse Reactions.

Unexpected Adverse Reaction (UAR)

An adverse reaction the nature and severity of which is not consistent with the information about the IMP in question set out in the SmPC.

Serious Adverse Event (SAE), Serious Adverse Reaction (SAR) or Suspected Unexpected Serious Adverse Reaction (SUSAR)

Any adverse event, adverse reaction or unexpected adverse reaction, respectively, that:

1. results in death, or
2. is life-threatening* (participant at immediate risk of death), or
3. requires in-patient hospitalisation or prolongation of existing hospitalisation**, or
4. results in persistent or significant disability or incapacity, or
5. consists of a congenital anomaly or birth defect, or
6. other important medical events that may not result in death, be life-threatening, incapacitating or requiring hospitalisation, but based upon appropriate medical judgement, may jeopardise the participant or may require medical or surgical intervention to prevent one of the other outcomes listed above

*'life-threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

**Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisations for a pre-existing condition, including elective procedures that have not worsened, do not constitute an SAE.

The assessment of seriousness of safety events should be performed by an appropriately delegated, medically qualified member of the site research team.

Serious Adverse Events are only expected to be reported up until visit 6.

10.3 Notes on Adverse Event Inclusions and Exclusions

10.3.1 Reference Safety Information

The Reference Safety Information (RSI) in TEMPEST is section 4.8 of the SmPC.

10.3.2 Include as an adverse event

- An exacerbation of a pre-existing illness
- An increase in frequency or intensity of a pre-existing episodic event/condition
- A condition (even though it may have been present prior to the start of the trial) detected after trial drug administration
- Continuous persistent disease or symptoms present at baseline that worsens following the administration of the study/trial treatment
- Laboratory abnormalities that require clinical intervention or further investigation (unless they are associated with an already reported clinical event).
- Abnormalities in physiological testing or physical examination that require further investigation or clinical intervention
- Injury or accidents

10.3.3 Do Not Include as an adverse event

- Medical or surgical procedures* - the condition which leads to the procedure is the adverse event
- Pre-existing disease or conditions present before treatment that do not worsen
- Situations where an untoward medical occurrence has occurred e.g. cosmetic elective surgery
- Overdose of medication without signs or symptoms**
- The disease being treated or associated symptoms/signs unless more severe than expected for the patient's condition

* *Cosmetic elective surgery is cited here as example of an event that is not reportable as an AE, however such an occurrence may need to be reported elsewhere in the CRF.*
** See also section 10.11.1. *If an overdose occurs and results in symptoms and signs that meet the criteria for adverse events as defined in Section 10.2, then they should be reported accordingly.*

10.3.4 Notification of deaths

All deaths must be reported to the LCTC within 24 hours of becoming aware using the SAE form.

10.3.5 Reporting of Pregnancy

Participants will be instructed through the PISC to immediately inform the investigator if they become pregnant during the trial and should be instructed to stop taking the IMP immediately. Pregnancies must be followed up until after the outcome.

Pregnancy and pregnancy follow-up should be recorded on the Pregnancy CRF. The Pregnancy CRF should be completed and submitted to the LCTC (who will inform the sponsor as per standard protocol) within 24 hours of learning of the pregnancy. LCTC will also inform Univar Solutions BV. Personal identifiable information will not be provided to Univar Solutions BV.

10.4 Notes on Severity / Grading of Adverse Events

The assignment of the severity/grading should be made by the investigator responsible for the care of the participant using the definitions below.
Regardless of the classification of an AE as serious or not, its severity must be assessed according to medical criteria alone using the following categories:

Mild: does not interfere with routine activities
Moderate: interferes with routine activities
Severe: impossible to perform routine activities

A distinction is drawn between serious and severe AEs. Severity is a measure of intensity (see above) whereas seriousness is defined using the criteria in section 10.2, hence, a severe AE need not necessarily be a Serious Adverse Event.

10.5 Relationship to Trial Treatment

The assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions in Table 2: Definitions of Causality.

Related: The AE follows a reasonable temporal sequence from administration of the IMP. It cannot reasonably be attributed to any other cause.
Not Related: The AE is probably produced by the participant's clinical state or by other modes of therapy administered to the participant.

If any doubt about the causality exists the local investigator should inform the study coordination centre who will notify the CI. In the case of discrepant views on causality between the investigator and others, the opinion of the treating investigator will never be downgraded and the MHRA will be informed of both points of view.

Table 2: Definitions of Causality

Relationship	Description
Unrelated	There is no evidence of any causal relationship. N.B. An alternative cause for the AE should be given
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).
Possibly	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments).
Probably	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Almost certainly	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

10.6 Expectedness

An AE whose causal relationship to the study drug is assessed by the investigator as "possible", "probable", or "definite" is an Adverse Drug Reaction.

All events judged by the investigator to be possibly, probably, or almost certainly related to the IMP, graded as serious and **unexpected** (see section 10.3 and SmPC for list of Expected Adverse Events) should be reported as a SUSAR.

Expectedness of AEs will be determined according to the summary of safety data provided in the SmPC. The CI or delegated other will provide an assessment of expectedness based on the data provided in the RSI section of the SmPC.

10.7 Procedures for recording adverse events

All AEs and ARs, and all SAEs, SARs and SUSARs should be reported to the Sponsor as per the relevant LCTC safety reporting procedure in accordance with the Medicines for Human Use Clinical Trials Regulations 2004 and amended Regulations 2006 and in accordance with the principles of GCP. The Investigator should also comply with the applicable regulatory requirements related to the reporting of SUSARs to the MHRA and REC. LCTC will also inform Univar Solutions BV. Personal identifiable information will not be provided to Univar Solutions BV.

For reported deaths, the site Principal Investigator should supply the Sponsor and the REC with any additional requested information (e.g. autopsy reports and terminal medical reports) as per the principles of GCP. This information will also be provided to Univar Solutions BV. Personal identifiable information will not be provided to Univar Solutions BV.

10.8 Follow-up after Adverse Events

All adverse events should be followed until satisfactory resolution or until the investigator responsible for the care of the participant deems the event to be chronic or the patient to be stable.
When reporting SAEs and SUSARs the investigator responsible for the care of the participant should apply the following criteria to provide information relating to event outcomes: resolved; resolved with sequelae (specifying with additional narrative); not resolved/ongoing; ongoing at final follow-up; fatal or unknown.

10.9 Reporting Procedures

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the LCTC in the first instance. A flowchart is given below to aid in determining reporting requirements.

10.9.1 Non-serious ARs/AEs

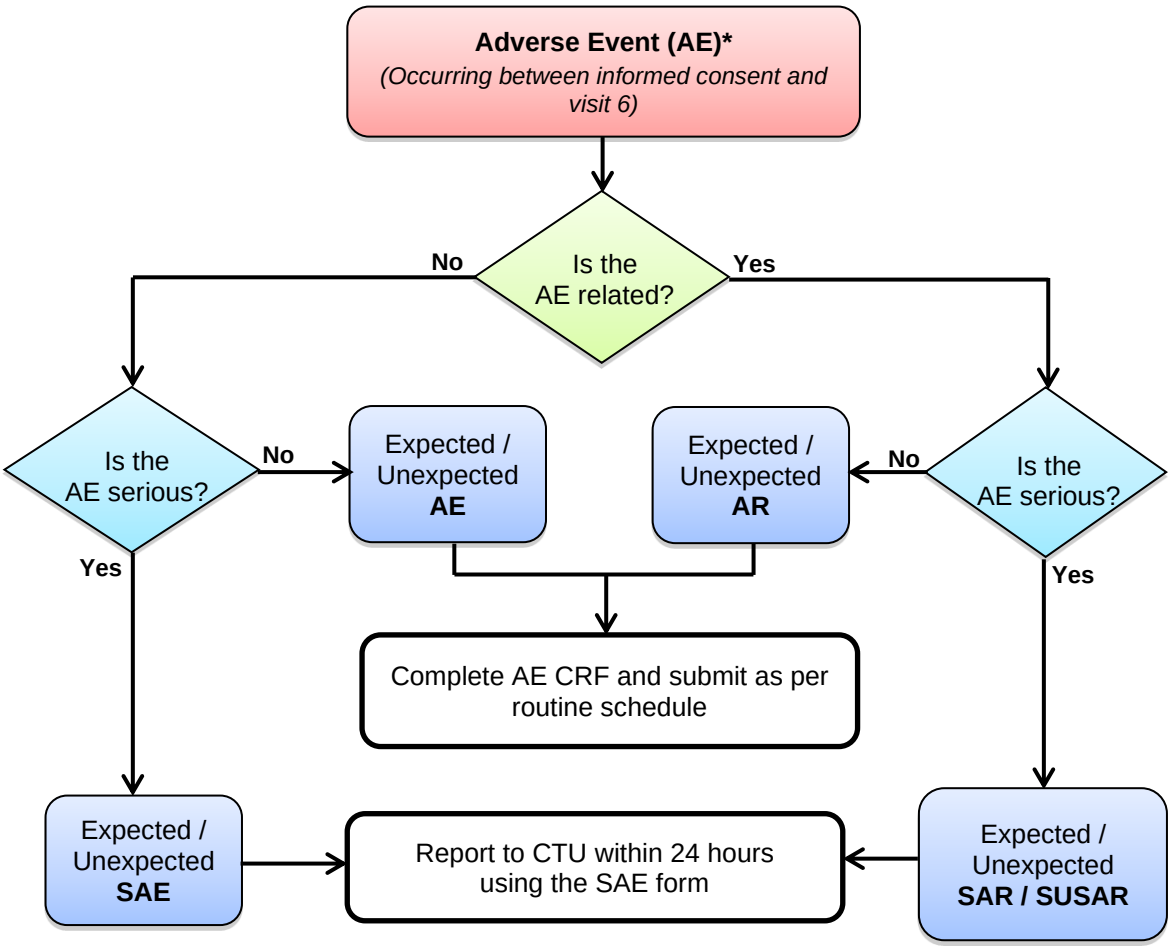
All such events, whether expected or not, should be recorded on an Adverse Event form, which should be transmitted to the LCTC within 7 days of the site team becoming aware of the AE.

10.9.2 Serious ARs/AEs/SUSARs

SARs, SAEs and SUSARs should be reported within 24 hours of the local site becoming aware of the event. The SAE form asks for the nature of event, date of onset, severity, corrective therapies given, outcome and causality. The responsible investigator should sign the causality of the event. Additional information should be sent within 5 days if the reaction has not resolved at the time of reporting.

The LCTC will notify the MHRA and REC of all SUSARs occurring during the study according to the following timelines: fatal and life-threatening within 7 days of notification; non-life threatening within 15 days. All investigators will be informed of all SUSARs occurring throughout the study. Local investigators should report any SUSARs and/or SAEs as required locally.

10.9.3 Flowchart for Reporting Requirements of Adverse Events



**If an adverse event occurs outside of this time window and the local investigator feels that the event is related to the trial treatment administered, the above process should still be followed.*

10.10 Responsibilities – Investigator

The Investigator is responsible for reporting all AEs that are observed or reported during the study, regardless of their relationship to study product. AEs should be actively monitored up until 24 hours after the end of study treatment.

All SAEs must be reported immediately by the investigator to the LCTC on an SAE form. All other adverse events should be reported on the regular progress/follow-up reports.

The SAE form will include, as a minimum: valid EudraCT number, sponsor trial number, one identifiable coded subject (randomisation number), one identifiable reporter (name of PI or appropriate delegated individual), one safety event, one suspect IMP (including active substance name-code) and a causality assessment.

1. The SAE form should be completed by a designated investigator, which is a physician named on the 'signature list and delegation of responsibilities log' as responsible for reporting SAEs and making trial related medical decisions. The investigator should assess the SAE for relatedness to the IMP. In the absence of the designated investigator the form should be completed and signed by an alternative member of the research site trial team and submitted to the LCTC. As soon as possible thereafter the responsible investigator should check the SAE form, make amendments as appropriate, sign and re-send to the LCTC. The initial report shall be followed by detailed reports as appropriate.

2. When submitting an SAE to the LCTC research sites should also telephone the appropriate trial co-ordinator to advise that an SAE report has been submitted.
3. Submit the SAE form (via secure email within 24 hours) to the LCTC.
4. The responsible investigator must notify their R&I department of the event (as per standard local governance procedures).
5. In the case of an SAE the participant must be followed-up until clinical recovery is complete and laboratory results have returned to normal, or until the event has stabilised. Follow-up may continue after completion of protocol treatment if necessary.
6. Follow-up information is noted on another SAE form by ticking the box marked 'follow-up' and transferring to the LCTC as information becomes available. Extra, annotated information and/or copies of test results may be provided separately.
7. The participant must be identified by trial number, date of birth and initials only. The participant's name should not be used on any correspondence.
8. Participants will be actively monitored for safety events up until Visit 6.

10.10.1 Maintenance of Blinding

This will be double blind study. The placebo and active treatments will appear identical and will be dispensed in identical containers with identical labelling. All trial participants, care providers, outcome assessors and data analysts will remain blind throughout the study.

Systems for SUSAR and SAR reporting should, as far as possible, maintain blinding of individual clinicians and of trials staff involved in the day-to-day running of the trial.

Unblinding clinicians may be unavoidable if the information is necessary for the medical management of particular participants. The safety of participants in the trial always takes priority. In each report, seriousness, causality and expectedness should be evaluated for all of the trial treatments unless criteria have been fulfilled (section 9.3) and unblinding has taken place.

Cases that are considered serious, unexpected and possibly, probably or almost certainly related to one of the trial therapies (i.e. possible SUSARs) would have to be unblinded at the LCTC prior to reporting to the regulator.

10.11 Responsibilities – LCTC

The LCTC is undertaking duties delegated by the trial sponsor, Manchester University NHS Foundation Trust, and is responsible for the reporting of SUSARs and other SARs to the regulatory authorities (MHRA, competent authorities of other European member states in which the trial is taking place and, if required, the research ethics committees (REC)) as follows:

- SUSARs which are fatal or life-threatening must be reported not later than 7 days after the LCTC is first aware of the reaction. Any additional relevant information must be reported within a further 8 days.
- SUSARs that are not fatal or life-threatening must be reported within 15 days of the LCTC first becoming aware of the reaction.
- A list of all SARs (expected and unexpected) must be reported annually.

It is recommended that the following safety issues should also be reported in an expedited fashion:

- An increase in the rate of occurrence or a qualitative change of an expected serious adverse reaction, which is judged to be clinically important;
- Post-study SUSARs that occur after the patient has completed a clinical trial and are notified by the investigator to the sponsor;

- New events related to the conduct of the trial or the development of the IMPs and likely to affect the safety of the participants, such as:
    a. A SAE which could be associated with the trial procedures and which could modify the conduct of the trial;
    b. A significant hazard to the participant population, such as lack of efficacy of an IMP used for the treatment of a life-threatening disease;
    c. A major safety finding from a newly completed animal study (such as carcinogenicity).
    d. Any anticipated end or temporary halt of a trial for safety reasons and conducted with the same IMP in another country by the same sponsor;
- Recommendations of the Data Monitoring Committee, if any, where relevant for the safety of the participants.

Staff at the LCTC will liaise with the CI (or designated other specified in the protocol) who will evaluate all SAEs received for seriousness, expectedness and causality. Investigator reports of suspected SARs will be reviewed immediately and those that are identified as SUSARs will be reported to regulatory authorities and REC. The causality assessment given by the local PI at an institution cannot be overruled and in the case of disagreement, both opinions will be provided with the report.

The PIs at all institutions participating in the trial will be notified of any SUSARs.

Patient safety incidents that take place in the course of research should be reported to the National Reporting and Learning System (NRLS) by each participating NHS Trust in accordance with local reporting procedures

10.11.1 Overdose

An overdose is defined as a known deliberate or accidental administration of trial medication. Overdoses may be observed from sources such as participant comments or pill counts. If an overdose occurs it should be reported by email to LCTC within 7 days and recorded on the study database. If an overdose results in symptoms and signs that meet the criteria for adverse events then they should be reported in the manner described in Section 10.

10.11.2 Safety reports

Safety reports will be generated during the course of the trial that allows for monitoring of SAE reporting rates across sites. The LCTC will send developmental safety update reports containing a list of all SARs to regulatory authorities and REC. Any concerns raised by the IDSMC or inconsistencies noted at a given site may prompt additional training at sites, with the potential for the LCTC to carry out site visits if there is suspicion of unreported AEs in patient case notes. Additional training will also be provided if there is unacceptable delay in safety reporting timelines. If any safety reports identify issues that have implications for the safety of trial participants, the PIs at all institutions participating in the trial will be notified.

10.11.3 Urgent Safety Measures

An urgent safety measure (USM) is a procedure not defined by the protocol, which is put in place prior to authorisation by the MHRA and REC in order to protect clinical trial participants from any immediate hazard to their health and safety.

If a USM is undertaken for TEMPEST, the LCTC in liaison with the study sponsor will notify MHRA and REC immediately and, in any event, within 3 days that such a measure has been taken and the reasons why it has been taken. The initial notification will be by telephone (ideally within 24 hours) and a notice in writing will be sent within 3 days, setting out the reasons for the USM and the plan for further action. After discussion with the MHRA and REC, further action will be agreed, which may include submission of a substantial amendment, a temporary halt, or permanent termination of the trial.

If TEMPEST is temporarily halted it will not recommence until authorised to do so by the MHRA and REC. If TEMPEST is permanently terminated before the date specified for its conclusion (see Section 8.9), the LCTC

will notify the MHRA and REC within 15 days of the date of termination by submitting the formal End of Trial Notification.

10.12 Treatment stopping rules

The trial may be prematurely discontinued by the Sponsor, CI or MHRA on the basis of new safety information or for other reasons given by the IDSMC, TSC, MHRA or REC concerned.

If the trial is prematurely discontinued, active participants will be informed. The MHRA and REC will be informed within 15 days of the early termination of the trial.

10.13 Contact Details and Out-of-hours Medical Cover

A 24-hour mobile phone number will be provided to participants. The phone will be managed by members of the research team who will have sufficient knowledge of the protocol and the IMP to be able to respond appropriately. In case the phone goes to voicemail, a voicemail message will clearly state to leave a message and expect a response within a given timescale.

11 STATISTICAL CONSIDERATIONS

11.1 Method of Randomisation

In this double-blind study, participants will be randomised to receive either trientine or placebo in a 1:1 ratio. Block randomisation, stratified by site, will be implemented, with computer generated randomisation allocations and randomly varying block sizes. The randomisation code will be generated by an independent LCTC statistician who is not involved with this trial.

11.2 Sample Size

Effect sizes, standard deviations (SD) and treatment discontinuation rates are based on the aforementioned pilot study of trientine in patients with HCM.[18]

11.2.1 Primary efficacy outcome

In the pilot study, the SD of within-patient differences in LVMi from baseline in the trientine group was 4.5g/m2 and in the observational control group was 2.4g/m2. Using a conservative SD of within-patient differences from baseline of 5g/m2 in both groups, 64 patients per group are required to detect a minimum difference, between trientine and placebo groups, of 2.5g/m2 in terms of change LVMi from baseline following 52 weeks of treatment (80% power, 5% significance level, 2-sided). To allow for treatment discontinuation in 25%, this is inflated to 86 per group i.e. total study 172.

11.2.2 Mechanistic outcomes

11.2.2.1 Extracellular mass

In the pilot study, the SD of within-patient differences in myocardial extracellular mass from baseline in the trientine group was 4.2g and in the observational control group was 3.4g. Using a conservative SD of within-patient differences from baseline of 4.5g in both groups, 64 patients per group provides 87% power to detect a minimum difference, between groups, of 2.5g in terms of change in myocardial extracellular mass from baseline following 52 weeks of treatment.

11.2.2.2 Cellular mass

In the pilot study, the SD of within-patient differences in cellular mass from baseline in the trientine group was 4.9g and in the observational control group was 4.1g. Using a conservative SD of within-patient differences from baseline of 5.0g in both groups, 64 patients per group provides 80% power to detect a minimum difference, between groups, of 2.5g in terms of change in cellular mass from baseline following 52 weeks of treatment.

11.2.2.3 PCr/ATP ratio (subgroup)

In pilot data from HCM patients undergoing baseline and follow-up 31P MRS (mean interval 6±2 years), using the same protocol as will be used here, the SD of within-patient differences in PCr/ATP ratio was 0.41. Using a conservative SD of within-patient differences from baseline of 0.55, 31 patients per group are required to detect an absolute minimum difference, between trientine and placebo groups, of 0.40 in terms of absolute change in PCr/ATP ratio from baseline following 52 weeks of treatment (80% power, 5% significance level, 2-sided). This effect size is based on that seen in other studies.[41] To allow for treatment discontinuation in 25%, this is inflated to 42 per group i.e. total subgroup size 84.

11.2.3 Feasibility

Sites have been chosen because they have large HCM clinical services, and investigators at these sites have expertise in the study procedures, have strong track records in HCM research and are active, enthusiastic clinical researchers. Recruitment audits have demonstrated that recruitment is feasible.

11.3 Statistical Analysis Plan

The trial will be analysed and reported using the 'Consolidated Standard of Reporting Trials' (CONSORT) and the ICH E9 guidelines. A full and detailed Statistical Analysis Plan (SAP) will be developed prior to any comparative analysis of the groups. The main features of the SAP are described briefly here.

Primary analysis will be by intention to treat (ITT), using complete case analysis, with a sensitivity regression analysis for the primary outcome (adjusting for variables which predict outcome to account for missingness).[42] An additional sensitivity analysis will estimate causal effect of treatment on the primary outcome by appropriately allowing for dose received using instrumental variable regression, thus accounting for informative premature treatment discontinuation.

LVMi (and other outcome measures) will be compared between groups using analyses of covariance, adjusting for baseline values. Correlation analysis will assess relationships between outcome parameters. A conventional 5% significance level will be used.

Potential mediators of treatment on the outcome (LVMi) include myocardial fibrosis (extracellular mass), cellular mass, PCr/ATP ratio and copper excretion. In order to test whether these variables predict change in LVMi, mediation analysis will be carried out adjusting for baseline covariates that predict both the mediator and LVMi. Sensitivity analyses will be conducted to assess the potential impact of unmeasured confounding between the mediator and outcome.

Changes in efficacy and mechanistic outcome measures will be assessed according to the most common HCM pathogenic variants, specifically beta-myosin heavy chain gene (MYH7), cardiac myosin-binding protein C (MYPBC3), cardiac troponin T (TNNT2), cardiac troponin I (TNNI3).

The following specific adverse events ("clinical endpoints" – see Section 3.5.4), including their corresponding dates where appropriate, will be compared individually and as a composite outcome between groups: death from cardiovascular causes, appropriate defibrillator discharge, aborted sudden death, heart transplant, progressive heart failure symptoms, new atrial fibrillation and septal reduction therapy.

11.4 Interim Monitoring and Analyses

There are no formal interim analyses planned.

11.4.1 IDSMC Review

Descriptive analyses of the accumulating data will be performed at regular intervals (at least annually) for review by an IDSMC. These analyses will be performed at the LCTC. The IDSMC will be asked to give advice on whether the accumulated data from the trial, together with results from other relevant trials, justifies continuing recruitment of further participants or further follow-up. A decision to discontinue recruitment, in all participants or in selected subgroups, will be made only if the decision is likely to convince a broad range of clinicians, participants in the trial and the general clinical community, or based on safety concerns. If a decision is made to continue, the IDSMC will advise on the frequency of future reviews of the data on the basis of accrual and event rates. The IDSMC will make recommendations to the TSC (see Section 15.4) as to the continuation of the trial.

12 REGULATORY AND ETHICAL APPROVALS

12.1 Statement of Compliance

Statement of compliance: The study will be carried out in accordance with:

- The principles of Good Clinical Practice
- The World Medical Association Declaration of Helsinki (1996),
- LCTC Liverpool Clinical Trials Centre SOPs
- The template content is structured consistent with the SPIRIT (Standard Protocol Item: Recommendations for Interventional Trials 2013)
- Policy Framework for Health and Social Care Research.

SI /EU Regulation	Title	Main impact/scope
2001/20/EC	The EU Clinical Trials Directive	National Competent Authority Ethics Framework GCP legal requirement Good Manufacturing Practice Protocol/Amendments/safety Protection of Vulnerable Groups Consent / Data protection
2004/1031	Medicines for Human use Clinical Trials Regulation	Transposed EU CT Directive in UK
2005/28/EC	EU Good Clinical Practice (GCP) Directive	Investigator brochure Archiving Mandatory training for trial teams
2006/1928	Amends 2004/1031	Investigator brochure /essential documents Serious Breach Declaration of Helsinki 1996 version for CTIMP
2006/2984	Amends 2004/1031	Consent for incapacitated adult by legal representative or emergency deferred consent
2008/941	Amends 2004/1031	Blood safety and quality Emergency Deferred consent for children
2009/1164	Miscellaneous Amendment	Urgent Safety measures
2009/3063	Amends 2004/1031	Nurse and pharmacists to prescribe unlicensed medicines

12.2 Regulatory Approval

This trial falls within the remit of the EU Directive 2001/20/EC, transposed into UK law as the UK Statutory Instrument 2004 No 1031: Medicines for Human Use (Clinical Trials) Regulations 2004 as amended. This trial has been registered with the MHRA and has been granted a Clinical Trial Authorisation (CTA).

12.3 Ethical Considerations

The study will abide by the principles of the World Medical Association Declaration of Helsinki (1964) and the Tokyo (1975), Venice (1983), Hong Kong (1989) and South Africa (1996). The study will also abide by the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the UK Policy Framework for Health and Social Care Research and the Medicines for Human Use (Clinical Trial) Regulations 2004, as amended in 2006 and any subsequent amendments.

Taking part in the study involves 6 visits to hospital.

Trientine has been used for nearly 50 years. It is safe and well tolerated. Potential side effects are listed in Section 3.2.1.

Study procedures are documented in Section 8.2. All are standard, non-invasive, well tolerated clinical procedures with minimal associated risk. All are performed as part of routine clinical management of patients with HCM except for ^{31}P MRS, although ^{31}P MRS is regularly performed for research purposes, is non-invasive, non-ionising and has minimal associated risk.

The research team explored the ethical considerations for the study with a dedicated Patient Advisory Group. The Group felt that the burden being placed on participants is acceptable, that the procedures involved are appropriate and that there are no major barriers to taking part.

12.4 Ethical Approval

This protocol and related documents will be submitted for review to an independent REC, and to the HRA for global governance approval.

Prior to opening a centre to recruitment, LCTC will ensure that local governance approval has been obtained: this will be “Capacity & Capability” Confirmation.

The CI will submit a final report at conclusion of the trial to the Sponsor, the REC and the MHRA within the timelines defined in the Regulations.

12.5 Protocol Deviation and Serious Breaches

A breach of the protocol or GCP is ‘serious’ if it meets the regulatory definition of being “likely to affect to a significant degree the safety or physical or mental integrity of the trial participants, or the scientific value of the trial”. All serious breaches of GCP or protocol will be reported to the MHRA and REC in an expedited manner by the LCTC.

If any persons involved in the conduct of the trial become aware of a potential serious breach, they must immediately report this to the LCTC who will in turn notify the sponsor within 24 hours. The sponsor will assess the breach and determine if is meets the criteria of a ‘serious’ breach of GCP or protocol and therefore requires expedited reporting to the MHRA and REC.

In determining whether or not the breach is likely to affect to a significant degree the safety, physical or mental integrity of participants, the sponsor may seek advice from medical expert members of the TMG and/or of the independent oversight committees (IDSMC and TSC). In determining whether or not the breach is likely to significantly affect the scientific value of the trial, the Sponsor may seek advice form the Trial Statistician. However, the sponsor retains responsibility for the assessment of whether or not a breach meets the definition of ‘serious’ and is subject to expedited reporting to MHRA and REC.

Breaches confirmed as ‘serious’ will be reported to the MHRA and REC within 7 days by the LCTC and notified to the TMG, IDSMC and TSC at their next meeting.

Any requests for additional information from the sponsor, TMG, TSC, IDSMC, REC or MHRA, will be promptly actioned by the relevant member(s) of the research team and open communication will be maintained to ensure appropriate corrective actions are taken and documented.
Incidence of protocol non-compliance are recorded as protocol deviations, the incidence of which are monitored and reported to trial oversight committees.

12.6 Study Discontinuation

In the event that the study is discontinued, participants will be treated according to usual standard clinical care. Participants who withdraw early from trial treatment but continue to allow follow-up as part of normal clinic visits will still have data collected as part of the trial. If participants withdraw from the trial completely, all data collected up until the time of withdrawal will be included in the study analysis. See Section 8.6 for further details.

13 DATA MANAGEMENT AND TRIAL MONITORING

Trial monitoring is carried out to ensure that the rights and well-being of participants are protected during the course of a clinical trial. A risk assessment will be performed for the trial, coordinated by the LCTC, in order to determine the level and type of monitoring required for specific hazards.

Monitoring can take the form of on-site visits or central monitoring. A detailed monitoring plan will be developed to describe who will conduct the monitoring, at what frequency monitoring will be done, and what level of detail monitoring will be conducted.

Details of the monitoring to be carried out for the TEMPEST study are included in the TEMPEST Trial Monitoring Plan.

Trial Oversight Committees related to the monitoring of the trial are detailed in section 15.4.

13.1 Risk assessment

In accordance with the relevant LCTC standard procedure, this trial has undergone a risk assessment, completed in partnership between:

1. Sponsor representative/s
2. CI
3. Co-ordinator and supervising trial managers
4. Trial statistician and supervising Statistician
5. Information Systems (IS) team
6. Data Management team
7. LCTC Director

In conducting this risk assessment, the contributors considered potential participant, organisational and study hazards, the likelihood of their occurrence and resulting impact should they occur.

The outcome of the risk assessment is expressed as a percentage, assigned according to the following categories:

1. Score ≤ 33% = Low risk
2. Score ≥34 to ≤ 67% = Moderate risk
3. Score ≥ 68 to ≤ 100% = High risk

This level of risk informs the risk assessment, regulatory requirements, nature and extent of the monitoring, and the management processes used in the trial. The score calculated for the TEMPEST trial indicate that it is a low risk trial.

13.2 Source Documents

Source documents will be as set out in the Source Document agreement.

13.3 Data Capture Methods

Electronic CRFs will be used.

SAE, pregnancy and unblinding forms must be returned to LCTC within 24 hours. AE forms must be returned to LCTC within 7 days of the form being due. All data should be entered into the study database in line with the timescales outlined in the eCRF completion guidelines.

A study database will be built and maintained by the LCTC using standard, secure clinical trial database software. Data will be populated into the database from the eCRFs. There will be a dedicated IS and a data manager for the study at the LCTC.

On all study-specific documents, other than the signed consent form, participants will be referred to using study participant numbers. Participants' names and any other identifying details will not be included.

All documents will be stored safely in secure, confidential conditions.

13.3.1 Electronic Case Report Forms (eCRF)

The study electronic case report form (eCRF) is the primary data collection instrument for the study. All data requested on the eCRF must be recorded.

13.4 Monitoring

13.4.1 Central Monitoring

Data stored at LCTC will be checked for missing or unusual values (range checks) and checked for consistency within participants over time. Any suspect data will be queried with the site via the database. Sites will respond to the queries providing an explanation/resolution to the discrepancy or by updating the data within the database. There are a number of monitoring features in place at the LCTC to ensure reliability and validity of the trial data, to be detailed in the trial monitoring plan.

13.4.2 Clinical Site Monitoring

In order to perform their role effectively, the trial coordinator (or monitor), sponsor monitor and persons involved in Quality Assurance and Inspection may need direct access to primary data, e.g. patient records, laboratory reports, appointment books, etc. Since this affects the patient's confidentiality, this fact is included on the PISC.

Direct access will be granted to authorised representatives from the Sponsor and regulatory authorities including the MHRA to permit study-related monitoring, audits and inspections.

The trial co-ordinator will monitor each site within 4 months of the first patient being randomised at that site.

13.5 Confidentiality

Individual participant medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited with the exceptions noted below.

eCRFs will be labelled with the participant's initials and unique trial screening and/or randomisation number. Medical information may be given to the participant's medical team and all appropriate medical personnel responsible for the participant's welfare.

The LCTC will be undertaking activities requiring the transfer of identifiable data.

Verification that appropriate informed consent is obtained will be enabled by the provision of copies of participant's signed informed consent forms being supplied to the LCTC by recruiting centres, which requires that name data will be transferred to the LCTC.

This transfer of identifiable data is disclosed in the PISC. The LCTC will preserve the confidentiality of participants taking part in the study and The University of Liverpool is registered as a Data Controller with the Information Commissioners Office. Sites should ensure that other trial documents are not posted in the same envelope as the consent form in order to reduce the risk to patient confidentiality.

The CI will act as custodian for the trial data. The following guidelines will be adhered to:

1. Patient data will be pseudo-anonymised.
2. Pseudo-anonymised data will be stored on a password-protected computer.
3. Trial data will be stored in line with the Medicines for Human Use (Clinical Trials) Regulations 2004 (and amendments) and the Data Protection Act and archived in line with the Medicines for Human Use (Clinical Trials) Regulations 2004 (and amendments) as defined in the relevant LCTC archiving procedure.

13.6 Quality Assurance and Quality Control

Quality Assurance (QA) includes all the planned and systematic actions established to ensure the trial is performed and data generated, documented/recorded and reported in compliance with applicable regulatory requirements. Quality Control (QC) includes the operational techniques and activities done within the QA system to verify that the requirements for quality of the trial-related activities are fulfilled e.g. state what clinical site monitoring (and audit) is planned, if any. In accordance with the monitoring plan, site visits will or will not be conducted and source verification performed if indicated to be required as a result of central monitoring processes. To this end, to ensure protocol compliance, ethical standards, regulatory compliance and data quality, the following will occur:

- The Trial Coordinator at the LCTC will verify appropriate approvals are in place prior to initiation of a site and the relevant personnel have attended trial specific training. A greenlight checklist will verify all approvals are in place prior to trial initiation at the LCTC and the individual site.
- The TMG will determine the minimum key staff required to be recorded on the delegation log in order for the site to be eligible to be initiated.
- Data will be evaluated for compliance with protocol and accuracy in relation to source documents.
- The study will be conducted in accordance with procedures identified in the protocol.
- Independent oversight of the trial will be provided by the Data and Safety Monitoring Committee and independent members of the TSC.
- The types of materials to be reviewed, who is responsible, and the schedule for reviews may be specified or referenced in other documents.
- Types and mechanisms of training of staff for the study should be specified.
- The PI and other key staff from each centre will attend site initiation training, coordinated by LCTC, which will incorporate elements of trial-specific training necessary to fulfil the requirements of the protocol.
- The TMG is to monitor screening, randomisation and consent rates between centres.
- The process for consent, recruitment and randomisation will be evaluated for compliance with the protocol.
- Data quality checks and monitoring procedures will be undertaken in line with the trial Data Management Plan.

13.7 Records Retention

The investigator at each investigational site must make arrangements to store the essential trial documents, (in compliance with the principles of GCP) the ISF and Pharmacy Site File, until the LCTC informs the investigator that the documents are no longer to be retained, or for a maximum period of 25 years (whichever is soonest).

In addition, the investigator is responsible for archiving of all relevant source documents so that the trial data can be compared against source data after completion of the trial (e.g. in case of inspection from authorities).

The investigator is required to ensure the continued storage of the documents, even if the investigator, for example, leaves the clinic/practice or retires before the end of required storage period. Delegation must be documented in writing.

The LCTC undertakes to store originally completed CRFs for the same period, except for source documents pertaining to the individual investigational site, which are kept by the investigator only. The LCTC will archive the documents. All electronic CRFs and trial data will be archived onto an appropriate media for long term accessible storage. Hard copies of data will be boxed and transferred to specially renovated, secure, premises where unique reference numbers are applied to enable confidentiality, tracking and retrieval.

14 INDEMNITY

TEMPEST is sponsored by Manchester University NHS Foundation Trust and co-ordinated by the LCTC in the University of Liverpool. The Manchester University NHS Foundation Trust does not hold insurance against claims for compensation for injury caused by participation in a clinical trial and they cannot offer any indemnity. As this is an investigator-initiated study, the Association of the British Pharmaceutical Industry (ABPI) guidelines for patient compensation by the pharmaceutical industry do not apply. However, in terms of liability, NHS Trust and Non-Trust Hospitals have a duty of care to patients treated, whether or not the patient is taking part in a clinical trial, and they are legally liable for the negligent acts and omission of their employees. Compensation is therefore available in the event of clinical negligence being proven.

Clinical negligence is defined as:

“A breach of duty of care by members of the health care professions employed by NHS bodies or by others consequent on decisions or judgments made by members of those professions acting in their professional capacity in the course of their employment, and which are admitted as negligent by the employer or are determined as such through the legal process”.

15 ROLES AND RESPONSIBILITIES

15.1 Role of Study Sponsor and Study Funder

Manchester University NHS Foundation Trust are the sponsoring organisation and are legally responsible for the trial. The sponsor delegates specific roles to the CI, LCTC and University of Oxford (UoO) with regards to trial design, conduct, data analysis and interpretation, manuscript writing, and dissemination of results. Delegations are described in the Collaboration Agreement.

15.2 Funding and Support in Kind

Funder	Financial and Non-financial Support Given
National Institute for Health Research (NIHR)	This project (project reference NIHR127575) is funded by the Efficacy and Mechanism Evaluation (EME) Programme, an MRC and NIHR partnership. The views expressed in this publication are those of the author(s) and not necessarily those of the MRC, NIHR or the Department of Health and Social Care. NIHR will monitor progress against key milestones via the submission of regular progress reports.

Univar is providing the trientine and placebo without charge.

NIHR and Univar have had no role in the study design other than through their external peer review process.

15.3 Protocol Contributors

Name	Affiliations	Contribution to protocol
Chris Miller	Manchester University NHS Foundation Trust and University of Manchester	Inception of trial, lead on the writing of this protocol, clinical and scientific arrangements, trial design and conduct
Emma Bedson	LCTC	Protocol development, governance arrangements and trial conduct
Carly Lawrence	LCTC	Protocol development, governance arrangements and trial conduct
Beatriz Duran	Manchester University NHS Foundation Trust	IMP management
Olatz Baroja	Manchester University NHS Foundation Trust	IMP management
Betty Raman	University of Oxford	Protocol development, trial design and conduct.
Masliza Mahmod	University of Oxford	Head of clinical trials, protocol development, trial design and conduct.
Hanan Lamlum	University of Oxford	Head of Clinical Research Governance and Coordination

		protocol development and conduct.
Ladislav Valkovic	University of Oxford	Senior MR Physicist
Stefan Neubauer	University of Oxford	Director of OCMR, Protocol development, trial design and conduct.
Matthias Schmitt	Manchester University NHS Foundation Trust	Protocol development, trial design and conduct.
John Farrant	Manchester University NHS Foundation Trust and University of Manchester	Protocol development, trial design and conduct.
Rob Cooper	Liverpool Heart and Chest Hospital NHS Foundation Trust	Protocol development, trial design and conduct.
Sanjay Prasad	Royal Brompton Hospital	Protocol development, trial design and conduct.
Clifford Garratt	Manchester University NHS Foundation Trust	Protocol development, trial design and conduct.
David Cotterell	Manchester University NHS Foundation Trust	Protocol development, trial design and conduct.
Suzanne Underhill	Manchester University NHS Foundation Trust	Protocol development, trial design and conduct.
Paula Smalley	Manchester University NHS Foundation Trust	Protocol development, trial design and conduct.
Josephine Naish	University of Manchester	Protocol development, trial design and conduct.
Chris Harrington	Royal Surrey County Hospital NHS Foundation Trust	Protocol development, trial design and conduct.
Susanna Dodd	LCTC	Protocol development, trial design and conduct, statistical oversight.

15.4 TRIAL COMMITTEES

15.4.1 Trial Management Group (TMG)

A TMG will be formed. Membership will include the CI, site investigators, patient representatives, Sponsor representative, pharmacy representative, members of the LCTC and representatives of other aspects of the study that are felt to be of be of benefit by being part of the TMG.

The TMG will be responsible for the day-to-day running and management of the trial and will meet monthly. The role of the TMG will include the following (refer to the TMG terms of reference and trial oversight committee membership document for further details):

1. Supervise the conduct and progress of the study, and adherence to the study protocol.
2. Assess the safety of the interventions during the study.
3. Evaluate the quality of the study data.
4. Review relevant information from other sources (e.g. related studies).
5. Escalate any issues for concern to the Sponsor, specifically where the issue could compromise patient safety or the integrity of the study or quality of the study data.

15.4.2 Trial Steering Committee (TSC)

The TSC will consist of an independent Chair, an independent statistician, an independent clinician with expertise relevant to the project, the CI and representatives from the TMG. An observer from the Sponsor and from the Funder will be invited to all meetings.

The role of the TSC is to provide overall supervision for the trial and provide advice through its independent Chair. The ultimate decision for the continuation of the trial lies with the TSC. It will meet at least annually.

The TSC will ensure the following (refer to the TSC Terms of Reference and Trial Oversight Committee membership document for further details):

1. Progress is satisfactory and the study is adhering to its overall objectives as set out in the protocol.
2. Patient safety is not being compromised.
3. The study is being conducted in accordance with the principles of GCP and the UK Clinical Trial Regulations.

Decisions about continuation or termination of the study or substantial amendments to the protocol will usually be the responsibility of the TSC, and the TSC will provide information and advice to the Sponsor, Funder and TMG in this regard.

15.4.3 Independent Data and Safety Monitoring Committee (IDSMC)

The IDSMC will consist of an independent Chair, an independent statistician and an independent clinician with expertise relevant to the project.

The IDSMC will be responsible for reviewing and assessing recruitment, interim monitoring of safety and effectiveness, trial conduct and external data. The IDSMC will first convene prior to the start of recruitment and will then define frequency of subsequent meetings (at least annually). Details of the interim analysis and monitoring are provided in section 11.4.

The IDSMC will provide a recommendation to the TSC concerning the continuation of the study. Refer to the IDSMC charter and Trial Oversight Committee membership document for further details.

16 PUBLICATION AND DISSEMINATION

16.1 Publication Policy

The results will be analysed and published as soon as possible. Individual investigators must undertake not to submit any part of their individual data for publication without the prior consent of the TMG.

The TMG will form the basis of the Writing Committee and advise on the nature of publications. The Uniform Requirements for Manuscripts Submitted to Biomedical Journals (http://www.icmje.org/) will be respected. All publications shall include a list of contributors, and if there are named authors, these should include the trial's CI, co-investigators, statistician(s) and trial manager(s) involved at least. If there are no named authors (i.e. group authorship) then a writing committee will be identified that would usually include these people, at least. The ISRCTN allocated to this trial should be attached to any publications resulting from this trial.

The members of the TSC and IDSMC should be listed with their affiliations in the Acknowledgements/Appendix of the main publication.

It is intended that the results of the study will be reported and disseminated at international conferences and in peer-reviewed scientific journals. The Sponsor publication procedure will be adhered to. Specifically, study results will be reported according to best practice, including CONSORT statements. Manuscripts will be reviewed by the trial statistician prior to submission for consideration of publication.

16.2 Dissemination to Key Stakeholders

The results of the trial will be disseminated as early as possible in order to appropriately inform policy and practice. This will include academic journal publications and presentations at academic conferences, Lay summaries of the study findings will be posted to the trial website and links to these summaries will be posted on patient group websites. Presentations will be made to patient groups and a symposium that brings together key stakeholders will be held.

16.3 Data Sharing

Individual investigators must not undertake to submit any part of their individual data for publication without the prior consent of the TMG.

17 CHRONOLOGY OF PROTOCOL AMENDMENTS

17.1 Version 2.0 (09/07/2020)

Summary of Amendments from Protocol V1.0 to Protocol V2.0		
Protocol Section Number	**Protocol Section Title**	**Summary of Changes**
N/A	Front page	Added CTA reference number Added REC reference number
7.1	Participant Identification and Screening	Wording amended to make it clear that only health care professionals can make the initial approach to the patient and not the research team.
9.3	Unblinding	Wording amended to make it clear that in the case of an emergency the decision to unblind a participant resides with the study investigators and the pharmacist should reveal the treatment allocation without approval from the CI and LCTC in this event. Added in section 9.3.4 Emergency unblinding to cover this.
10.1	Time Period for Safety Reporting	Wording amended to state that serious adverse events or reactions will be reported from the time of informed consent, instead of from randomisation.
10.9.3	Flowchart for Reporting Requirements of Adverse Events	Wording amended to state that serious adverse events or reactions will be reported from the time of informed consent, instead of from randomisation.
15.2	Funding and Support in Kind	Funder reference number corrected.
N/A	N/A	Removal of references to ICH GCP throughout protocol and replaced with Principles of GCP to ensure consistency.

17.2 Version 1.0 (14/05/2020)

Original version.

18 REFERENCES

1. Semsarian C, Ingles J, Maron MS, et al. New perspectives on the prevalence of hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2015;65:1249-1254.
2. Olivotto I, Maron MS, Adabag AS, et al. Gender-Related Differences in the Clinical Presentation and Outcome of Hypertrophic Cardiomyopathy. *JAC*. 2005;46:480-487.
3. Maron MS, Olivotto I, Zenovich AG, et al. Hypertrophic cardiomyopathy is predominantly a disease of left ventricular outflow tract obstruction. *Circulation*. 2006;114:2232-2239.
4. Rowin EJ, Maron BJ, Kiernan MS, et al. Advanced heart failure with preserved systolic function in nonobstructive hypertrophic cardiomyopathy: under-recognized subset of candidates for heart transplant. *Circ: Heart Fail*. 2014;7:967-975.
5. Harris KM, Spirito P, Maron MS, et al. Prevalence, clinical profile, and significance of left ventricular remodeling in the end-stage phase of hypertrophic cardiomyopathy. *Circulation*. 2006;114:216-225.
6. Authors/Task Force m, Elliott PM, Anastasakis A, et al. 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy. *Eur Heart J*. 2014;35:2733-79.
7. Gersh BJ, Maron BJ, Bonow RO, et al. 2011 ACCF/AHA guideline for the diagnosis and treatment of hypertrophic cardiomyopathy. *Circulation*. 2011;124:2761-96.
8. Force T, Bonow RO, Houser SR, et al. Research Priorities in Hypertrophic Cardiomyopathy: Report of a Working Group of the National Heart, Lung, and Blood Institute. *Circulation*. 2010;122:1130-1133.
9. Spoladore R, Maron MS, D'Amato R, et al. Pharmacological treatment options for hypertrophic cardiomyopathy: high time for evidence. *Eur Heart J*. 2012;33:1724-1733.
10. Cooper GJ, Chan YK, Dissanayake AM, et al. Demonstration of a hyperglycemia-driven pathogenic abnormality of copper homeostasis in diabetes and its reversibility by selective chelation: quantitative comparisons between the biology of copper and eight other nutritionally essential elements in normal and diabetic individuals. *Diabetes*. 2005;54:1468-76.
11. Cooper GJS, Phillips ARJ, Choong SY, et al. Regeneration of the heart in diabetes by selective copper chelation. *Diabetes*. 2004;53:2501-2508.
12. Lu J, Pontr B, Pickup S, et al. Treatment with a copper-selective chelator causes substantive improvement in cardiac function of diabetic rats with left-ventricular impairment. *Cardiovascular diabetology*. 2013;12:1-1.
13. Cooper GJS. Therapeutic Potential of Copper Chelation with Triethylenetetramine in Managing Diabetes Mellitus and Alzheimer's Disease. *Drugs*. 2011;71:1281-1320.
14. Gong D, Lu J, Chen X, et al. Molecular changes evoked by triethylenetetramine treatment in the extracellular matrix of the heart and aorta in diabetic rats. *Mol Pharmacol*. 2006;70:2045-51.
15. Gong D, Lu J, Chen X, et al. A copper(II)-selective chelator ameliorates diabetes-evoked renal fibrosis and albuminuria, and suppresses pathogenic TGF-beta activation in the kidneys of rats used as a model of diabetes. *Diabetologia*. 2008;51:1741-51.
16. Cooper GJ, Young AA, Gamble GD, et al. A copper(II)-selective chelator ameliorates left-ventricular hypertrophy in type 2 diabetic patients: a randomised placebo-controlled study. *Diabetologia*. 2009;52:715-22.
17. Potluri R, Roberts N, Miller CA, et al. Impaired copper homeostasis in patients with hypertrophic cardiomyopathy. *Eur Heart J*. 2012;33(suppl_1):P4294.
18. Reid A, Miller CA, Cooper GJS, et al. Copper chelation in hypertrophic cardiomyopathy; open-label pilot study assessing the effects of trientine on LV mass, myocardial fibrosis, performance and metabolism. *Eur Heart J*. 2017;38 (Suppl):P4503.
19. Jüllig M, Chen X, Hickey AJ, et al. Reversal of diabetes-evoked changes in mitochondrial protein expression of cardiac left ventricle by treatment with a copper(II)-selective chelator. *PROTEOMICS – Clinical Applications*. 2007;1:387-399.
20. Ashrafian H, Redwood C, Blair E, et al. Hypertrophic cardiomyopathy:a paradigm for myocardial energy depletion. *Trends Genet*. 2003;19:263-8.
21. Crilley JG, Boehm EA, Blair E, et al. Hypertrophic cardiomyopathy due to sarcomeric gene mutations is characterized by impaired energy metabolism irrespective of the degree of hypertrophy. *J Am Coll Cardiol*. 2003;41:1776-82.
22. Raman B, Ariga R, Spartera M, et al. Progression of myocardial fibrosis in hypertrophic cardiomyopathy: mechanisms and clinical implications. *European heart journal cardiovascular Imaging*. 2018.
23. Frey N, Luedde M and Katus HA. Mechanisms of disease: hypertrophic cardiomyopathy. *Nat Rev Cardiol*. 2012;9:91-100.
24. Condamine L, Hermine O, Alvin P, et al. Acquired sideroblastic anaemia during treatment of Wilson's disease with triethylene tetramine dihydrochloride. *Br J Haematol*. 1993;83:166-8.
25. Perry AR, Pagliuca A, Fitzsimons EJ, et al. Acquired sideroblastic anaemia induced by a copper-chelating agent. *Int J Hematol*. 1996;64:69-72.

26. Brewer GJ, Askari F, Lorincz MT, et al. Treatment of Wilson disease with ammonium tetrathiomolybdate: IV. Comparison of tetrathiomolybdate and trientine in a double-blind study of treatment of the neurologic presentation of Wilson disease. *Arch Neurol*. 2006;63:521-7.
27. Dahlman T, Hartvig P, Lofholm M, et al. Long-term treatment of Wilson's disease with triethylene tetramine dihydrochloride (trientine). *QJM*. 1995;88:609-16.
28. Bellenger N and Davies L. Reduction in sample size for studies of remodeling in heart failure by the use of cardiovascular magnetic resonance. *J Cardiovasc Magn Reson*. 2000;2:271-278.
29. Miller CA, Naish JH, Bishop P, et al. Comprehensive Validation of Cardiovascular Magnetic Resonance Techniques for the Assessment of Myocardial Extracellular Volume. *Circulation: Cardiovascular Imaging*. 2013;6:373-383.
30. Flett AS, Hayward MP, Ashworth MT, et al. Equilibrium contrast cardiovascular magnetic resonance for the measurement of diffuse myocardial fibrosis: preliminary validation in humans. *Circulation*. 2010;122:138-144.
31. White SK, Sado DM, Fontana M, et al. T1 mapping for myocardial extracellular volume measurement by CMR: bolus only versus primed infusion technique. *JACC Cardiovasc Imaging*. 2013;6:955-62.
32. Schelbert EB, Testa SM, Meier CG, et al. Myocardial extravascular extracellular volume fraction measurement by gadolinium cardiovascular magnetic resonance in humans: slow infusion versus bolus. *J Cardiovasc Magn Reson*. 2011;13:16.
33. Kawel N, Nacif M, Zavodni A, et al. T1 mapping of the myocardium: intra-individual assessment of post-contrast T1 time evolution and extracellular volume fraction at 3T for Gd-DTPA and Gd-BOPTA. *J Cardiovasc Magn Reson*. 2012;14:26.
34. Wong TC, Piehler KM, Kang IA, et al. Myocardial extracellular volume fraction quantified by cardiovascular magnetic resonance is increased in diabetes and associated with mortality and incident heart failure admission. *Eur Heart J*. 2014;35:657-64.
35. Evin M, Cluzel P, Lamy J, et al. Assessment of left atrial function by MRI myocardial feature tracking. *J Magn Reson Imaging*. 2015;42:379-89.
36. Gertz RJ, Lange T, Kowallick JT, et al. Inter-vendor reproducibility of left and right ventricular cardiovascular magnetic resonance myocardial feature-tracking. *PLoS One*. 2018;13:e0193746.
37. Prasad SK and Lota AS. Improving Risk Stratification by Cardiac Magnetic Resonance Imaging in Heart Failure: Is Strain the Missing Link? *JACC Cardiovasc Imaging*. 2018;11:1430-1432.
38. Schulz-Menger J, Bluemke DA, Bremerich J, et al. Standardized image interpretation and post processing in cardiovascular magnetic resonance: Society for Cardiovascular Magnetic Resonance (SCMR) board of trustees task force on standardized post processing. *J Cardiovasc Magn Reson*. 2013;15:35.
39. American Thoracic S and American College of Chest P. ATS/ACCP Statement on cardiopulmonary exercise testing. *Am J Respir Crit Care Med*. 2003;167:211-77.
40. Dass S, Cochlin LE, Suttie JJ, et al. Exacerbation of cardiac energetic impairment during exercise in hypertrophic cardiomyopathy: a potential mechanism for diastolic dysfunction. *Eur Heart J*. 2015.
41. Abozguia K, Elliott P, McKenna W, et al. Metabolic Modulator Perhexiline Corrects Energy Deficiency and Improves Exercise Capacity in Symptomatic Hypertrophic Cardiomyopathy. *Circulation*. 2010;122:1562-1569.
42. Jakobsen J, Gluud C, Wetterslev J et al. When and how should multiple imputation be used for handling missing data in randomised clinical trials – a practical guide with flowcharts. *BMC Medical Research Methodology*. 2017;17:162 https://doi.org/10.1186/s12874-017-0442-1

19 DOCUMENTS SUPPLEMENTARY TO THE PROTOCOL

Documents referenced within the protocol are separately maintained and version controlled. Any of the supplementary documents subject to MHRA and / or ethical review are submitted as separate version controlled documents.

20 APPENDICES