STUDY PROTOCOL

Inflammation and Salt-inducible kinases - A Potential Novel Therapeutic Strategy in Patients with Heart Failure

Study Acronym	SIK-HF
Sponsor	University of Dundee
Sponsor Number	2-012-22
Funder	Tenovus
Chief Investigator	Dr Ify Mordi
REC Number	To be confirmed
ISRCTN Number	To be confirmed
Version Number and Date	1: 13/04/2022

PROTOCOL APPROVAL

The undersigned confirm that the following protocol has been agreed and approved by the Sponsor and that the Chief Investigator agrees to conduct the study in compliance with this approved protocol and will adhere to the principles of GCP, the Sponsor SOPs, and any other applicable regulatory requirements as may be amended from time to time.

Dr Ify Mordi		13/04/2022
Chief Investigator	Signature	

LIST OF ABBREVIATIONS

AE	Adverse Event
CI	Chief Investigator
CANTOS	Canakinumab Anti- inflammatory Thrombosis Outcome Study
CRF	Case Report Form
CTIMP	Clinical Trials of Investigational Medicinal Products
DMC	Data Monitoring Committee
GCP	Good Clinical Practice
HF	Heart Failure
HFrEF	Heart failure with reduced ejection fraction.
HFpEF	Heart failure with preserved ejection fraction
HRA	Heath Research Authority
hsCRP	high-sensitivity C-reactive protein
ICF	Informed Consent Form
IF	Incidental Findings
IL-1B	Interleukin 1 beta
IL-6	Interleukin 6

ISF	Investigator Site File
LPLV	last patient last visit
LVEF	Left ventricle ejection fraction
LVH	Left ventricle hypertrophy
NCTIMPS	Non-Clinical Trials of Investigational Medicinal Products
PI	Principal Investigator
QoL	Quality of Life
TNF	Tumour necrosis factor
TASC	Tayside Medical Science Centre
REC	Research Ethics Committee
SAE	Serious Adverse Event
SD	Standard Deviation
SIKs	Salt inducible Kinases
SPSS	Statistical Package for the Social Sciences
SMF	Study Master File
SMG	Study Management Group

STUDY SUMMARY

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Study Title	Inflammation and Salt-inducible kinases - A potential Novel therapeutic strategy in patients with heart failure	
Study Design	Prospective, observational	
Study Population	Adults (aged ≥18 years) with a diagnosis of heart failure (either stage B or stage C)	
Sample Size	100	
Study Period	18 months	
Clinical phase duration	18 months	
Follow up phase duration	None	
	Objectives 1. To determine whether levels of systemic inflammation and SIK activity are linked to severity of heart failure, providing clinical evidence for the role of SIK-related inflammation in HF. 2. To assess whether inhibition of SIK activity is associated with a reduction in the markers of inflammation in HF.	Outcome Measures 1. Correlations between measures of systemic inflammation and SIK activity with cardiac structural and functional parameters and quality of life in HF patients. 2. To determine, in an ex-vivo experiment on blood from HF patients, whether, SIK inhibition can reduce levels of inflammatory cytokines.

Inclusion Criteria	1. Adults (>18 years) 2. Diagnosis of heart failure (Stage B or C) as judged by the treating clinician.
Exclusion Criteria	Unable or unwilling to give consent

1. **INTRODUCTION**

Heart failure (HF) is a complex clinical syndrome which results from any disorder that leads to impairment in ventricular filling or ejection of blood to the systemic circulation[1]. It often causes multiple debilitating symptoms, such as fatigue, pain, peripheral oedema, and breathlessness. Heart failure is categorized according to left ventricular ejection fraction (LVEF) into heart failure with reduced ejection fraction (HFrEF) (usually considered LVEF 40% or less) and heart failure with preserved ejection fraction (HFpEF), variably considered as LVEF >40-50%.[1] Systemic inflammation has been shown to be implicated and highly prevalent in both heart failure with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF), via comorbidities such as diabetes and obesity and underlying pro-inflammatory substrates such as endothelial dysfunction and atherosclerosis [2]. Interleukins 1β and 6 (IL-1β and IL-6), pro-inflammatory cytokines that are regulated by salt-inducible kinases (SIKs), are increased in patients with HF.[3] Studies have shown that inhibition of SIKs also increases anti-inflammatory cytokines such as IL-10.[4,5] A recent study revealed that inhibition of SIKs was associated with attenuation of adverse remodelling (particularly left ventricular hypertrophy (LVH) and fibrosis), a precursor to the development of heart failure[6]. Following these studies, my project will investigate heart failure, cardiac structural, and functional parameters responses to SIK activity.

2. BACKGROUND & RATIONALE

Heart failure (HF) is a global public health burden.[7] It is estimated that 64.3 million people are living with heart failure worldwide[8] . In the UK alone,1 million people are thought to be living with HF. Despite advances in treatment of HFrEF over the past 30 years it is still associated with a substantial mortality and morbidity. Of more considerable concern, however, is the lack of evidence-based treatments for HFpEF. HFpEF accounts for almost half of all HF cases and is increasing in prevalence. This lack of any evidence-based treatments represents a critical unmet need which needs to be addressed urgently.

Inflammation and inflammatory cytokines have been shown to play an important role in pathophysiology of the heart failure. Tumour necrosis factor alpha (TNFα) and interleukins 1β and 6 (IL-1β and IL-6), pro-inflammatory cytokines, are increased in patients with HF[3]. Upregulation of these cytokines is driven by the innate immune system activated by pattern recognition receptors such as Toll-like receptors in the myocardium[9]. This activation leads to an increase in pro-inflammatory

cytokine expression and activation of humoral immunity (B and T cells).These pro-inflammatory cytokines, in animal studies, have been associated with negative inotropic effects[9,10], adverse left ventricular remodelling[11,12] (preludes to development of HF) and increased myocardial fibrosis[13], providing evidence for the causal role of inflammation in HF. Left ventricular adverse remodelling in response to pressure overload is attenuated by deletion of pro-inflammatory cytokines gene such as IL-6 , again, suggesting a causal role for pro-inflammatory cytokines in HF.[14] Adverse left ventricular remodelling (e.g. left ventricular hypertrophy or left atrial enlargement) can be present before the typical symptoms of HF (such as breathlessness or ankle oedema) develop – this is known as stage B HF (patients with HF symptoms are classified as stage C HF). In patients with heart failure, the levels of these pro-inflammatory cytokines are significantly increased. Notably, they are associated with worse symptom status and quality of life (QoL). Deterioration in patients symptoms is associated with increases in levels of inflammation.[15] High levels of inflammatory cytokines have also been associated with worse prognosis in patients with HFrEF and HFpEF, although relationships with adverse remodelling in HF are less well investigated.[16,17]

Current guideline-recommended treatment for heart failure (renin-angiotensin-aldosterone system blockers and beta-blockers), appear to have, at best, a marginal effect on inflammation[18,19], thus there may still be a role for therapies targeting inflammation to improve outcome in HF. Recently, the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) has shown promising data that suggest therapy with IL-1β inhibitor canakinumab is related to a dose-dependent reduction in hospitalisation for HF (HHF) and the composite of HHF or HF–related mortality compared to placebo in patients with a previous myocardial infarction and elevated high-sensitivity C-reactive protein (hsCRP), irrespective of the presence of HF at baseline[20]. An increase in level of IL-6 and hsCRP was significantly associated with raised risk of HF while lowering of hsCRP following canakinumab therapy was associated with a significant decrease in the risk of combined outcome. These observations raise the possibility that lowering the levels of inflammation, particularly in heart failure patients with high basal systemic inflammation, could be a strategy for better outcomes. Finding the correct mechanistic targets could provide a new avenue to improve the outcomes of patients with HF.

Recently, salt-inducible kinases have been shown to play a critical role in HF[21]. In addition to several roles, SIKs play a critical part in regulating inflammation through the nuclear factor-KB pathway and cAMP-regulated transcriptional co-activator phosphorylation [22]. Suppression of SIKs result in down-

regulation of pro-inflammatory cytokines including IL-1 and IL-6[21] and increase in anti-inflammatory cytokines such as IL-10 – in this sense, SIKs could be thought of as master regulators of inflammation.[4,5]

There is some evidence to suggest that SIKs may play a role in the pathophysiology of HF. In an animal study, inhibition of SIKs was associated with attenuation of adverse left ventricular remodelling (which is the leading cause of HF)[6]. In the study, the expression of genes associated with left ventricular hypertrophy (LVH) and an increase in left ventricle wall thickness, has been shown to increase in SIK2+/+ but not in SIK2-/- mice in response to a high-salt diet[23]. In spite of this promising pre-clinical data, to the best of our knowledge, there has not been any study on SIK activity or inhibition in patients with heart failure. What is now needed is a mechanistic clinical study in patients with heart failure to bridge the translational gap between pre-clinical data that has established the role of SIKs and pro-inflammatory cytokines in heart failure and provide evidence to support future clinical trials to determine if targeting SIKs in HF patients could provide a novel therapeutic strategy for treatment of HF.

3. THE AIMS OF THE STUDY

1. To relate measures of systemic inflammation and SIK activity with cardiac structural and functional parameters and quality of life in HF patients.

2. To assess, in an ex-vivo experiment on blood from HF patients, whether SIK inhibition can reduce levels of inflammatory cytokines.

4. THE OBJECTIVES AND OUTCOMES OF THE STUDY

1. To determine whether levels of systemic inflammation and SIK activity are related to cardiac structural, functional parameters, and quality of life in patients with heart failure.

Hypothesis: Higher levels of systemic inflammation are associated with greater severity of HF, judged by more cardiac structural and functional abnormalities and worse quality of life than those with lower levels of inflammation.

2. To assess, in an ex-vivo study in patients with HF, whether SIK inhibition is associated with a reduction in inflammation, potentially supporting this as a novel therapeutic strategy in patients with HF.

Hypothesis: SIK inhibition is associated with a reduction in markers of inflammation in HF patients

We will conduct a prospective, observational pilot cohort study and recruit 100 patients with stage B or C HF. Stage B includes patients with structural heart disease but without signs and symptoms of HF while stage C encompasses patients who have structural heart disease with prior or current symptoms of HF. We will take blood samples at baseline and 6 months and perform quantitative assessment of inflammatory cytokines (neutrophil/lymphocyte ratio, IL-1β, IL-6, IL-10, TNF-α, E-selectin, sICAM-1, high-sensitivity C-reactive protein). We will perform echocardiography and assess the quality of life using the Kansas City Cardiomyopathy Questionnaire (KCCQ) and correlate the parameters with the inflammatory markers.

The primary outcome of our study will be SIK activity and inflammatory gene expression measured from peripheral blood mononuclear cells.

5. STUDY DESIGN

5.1 Study Description

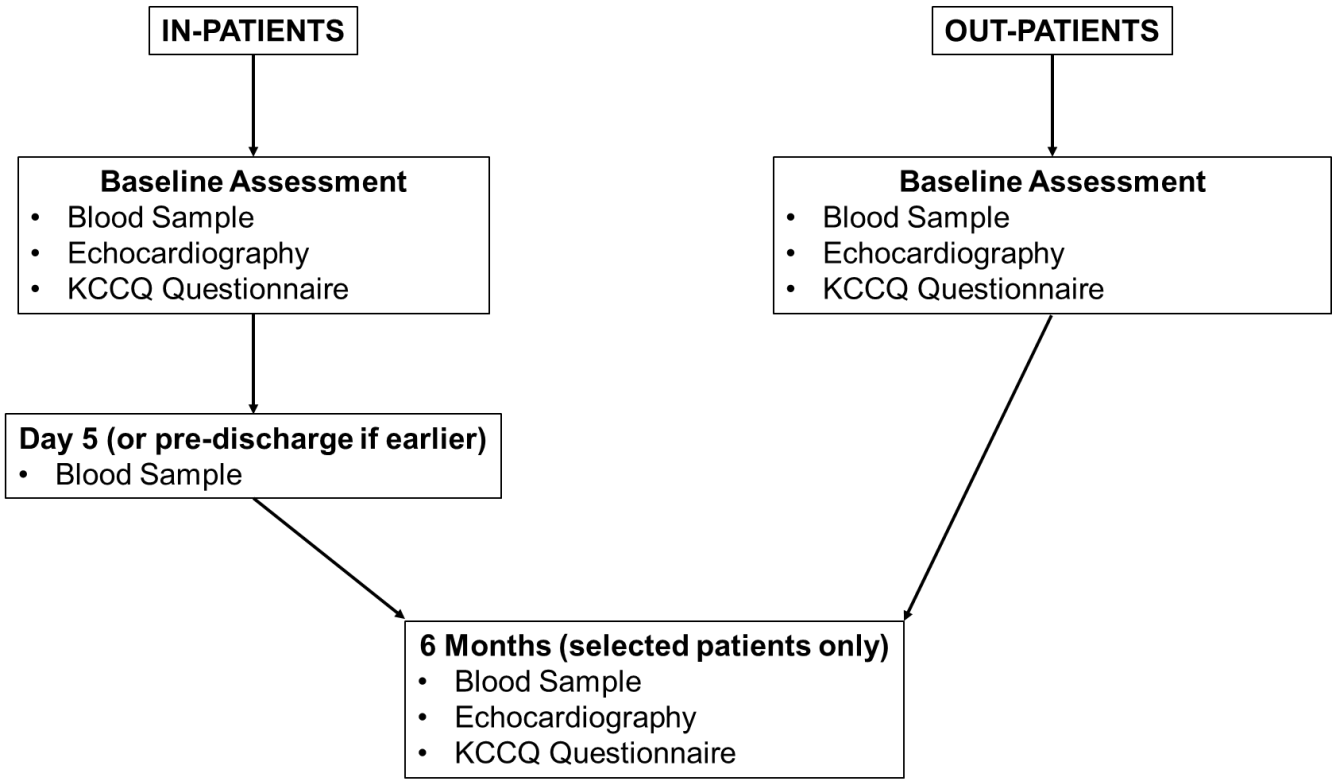
We will conduct a prospective, observational pilot cohort study of 100 patients with stage B or C HF. As this is a pilot study, we will recruit a spread of patients based on echocardiographic left ventricular ejection fraction to include both HF with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF), however, no more than 70 of either HFrEF or HFpEF will be recruited.

We will recruit participants from both the in-patient and out-patient settings. For in-patients, permission to approach patients to take part in the study will be sought from the clinical cardiology team who will give the patient information sheet to the patient before any members of the research team contact them. Out-patients will be recruited from cardiology clinics, again with permission of the treating cardiologist who will approach the patient first.

All patients will have a blood sample taken at baseline, alongside the Kansas City Cardiomyopathy Questionnaire (KCCQ; for assessment of quality of life) and an

echocardiogram. For in-patients, a second blood sample will be taken, either at 5 days or pre-discharge (if earlier than 5 days). We will recall 40 patients for a repeat blood sample, KCCQ and echocardiography at 6 months. We aim to recall participants with the highest and lowest levels of inflammation at baseline (measured by neutrophil:lymphocyte ratio).

5.2 Study Flowchart



5.3 Study Matrix

	Baseline (n=100)	**Day 5 (or pre-discharge if earlier) – IN-PATIENTS ONLY**	**6 months (n=40)**
Informed Consent	X		
Blood sample	X	X	X
Echocardiography	X		X
KCCQ Questionnaire	X		X

5.4 Study Assessments

In all patients assessments (blood sample, echocardiography and KCCQ questionnaire) will be undertaken at baseline (within 48 hours of admission for in-patients). Activities will take place either on the cardiology ward (for in-patients who are unable to leave the ward) or the Division of Molecular and Clinical Medicine, Ninewells Hospital. Patients recruited from the in-patient setting will also be asked to provide an additional blood sample at day 5 (or on the day of discharge if earlier). We will recall 40 patients for a further assessment at 6 months, selecting those with the highest and lowest levels of inflammation at baseline (measured by neutrophil:lymphocyte ratio - NLR). We have previously shown that a decrease in inflammation NLR at 6 months is associated with improved outcome.[24]

Blood sampling

A blood sample will be taken from patients by a delegated member of the study research team of the study, by venepuncture from vein via a butterfly needle and evacuated sample tubes. This blood sample (approximately 50ml) will be drawn in EDTA-treated tubes. Samples will be centrifuged, and plasma from the sample will immediately be transferred into aliquots. The samples will be stored in a -80°C freezer. This will be performed in the Division of Molecular and Clinical Medicine, Ninewells Hospital, University of Dundee, with analysis done in batches over the duration of the study. Surplus blood will be stored in freezers in the Division of Molecular and Clinical Medicine, Ninewells Hospital.

Sample analysis: Characterisation of SIK expression (protein, mRNA, enzyme activity) will be determined by quantitative polymerase chain reaction (qPCR), immunoblotting (protein expression) and IP-kinase assay (enzymatic activity) as described previously.[25]

Samples will also be analysed for inflammatory markers including IL-1β, IL-6, IL-10, TNF-α, E-selectin, sICAM-1 and high-sensitivity C-reactive protein using Bio-Plex® Precision ProkitsTM (BIO-RAD laboratories). We will also measure neutrophil/lymphocyte ratio and isolate peripheral blood mononuclear cells (PBMCs) from blood samples.

In an ex-vivo experiment we will stimulate PBMCs with oxidised LDL, measuring the levels of inflammatory cytokine expression in the presence and absence of clinically approved SIK inhibitors such as bosutinib and dasatinib.

Echocardiography

Transthoracic echocardiography will be performed at baseline and at 6 months. A detailed 2-dimensional transthoracic echocardiographic assessment will be performed as per European Society of Cardiovascular Imaging standard guidelines.[26] This will include 2-dimensional and Doppler imaging for assessment of cardiac structure and function.

Quality of Life (QoL) Assessment

Quality of life will be assessed at baseline and 6 months using the Kansas City Cardiomyopathy Questionnaire, a validated tool for measuring QoL in heart failure patients.[27] We will use the full 23-item questionnaire to assess both the overall and clinical summary scores.

Clinical Outcomes

We will collect data on death and hospitalisations during the duration of the study.

5.5 Study safety assessments

No specific safety assessments will be performed.

COVID-19

No increased risk of COVID-19 to staff or participants is anticipated from this study. Local and national clinical guidelines at the time will be followed. Standard mitigation will be performed (mask-wearing, distancing where feasible). If guidance restricts out-patient activity then we will focus recruitment on in-patients.

5.6 Tissue

Blood samples will be taken as per section 5.4. Surplus blood will be stored for future research in the Division of Molecular and Clinical Medicine and registered with the Tayside Biorepository.

5.7 Incidental findings

Any incidental findings considered to be clinically significant will be reported to the participant's GP and/or consultant by the CI or Site PI, with the consent of the participant.

5.8 Study population

All patients aged ≥18 years with a diagnosis of HF will be eligible to take part in this study.

5.9 Number of participants

We expect to recruit 100 patients in total. As an observational study, we do not expect any dropout. Recruitment will be carried out over 12 months.

5.10 Inclusion criteria

1. Adults (>18 years)
2. Diagnosis of heart failure (stage B or stage C).
3. Able to give informed consent.

5.11 Exclusion criteria

Unable or unwilling to consent.

Individuals will not be enrolled to the study if they are participating in the clinical phase of another interventional trial/study or have done so within the last 30 days.

6. PARTICIPANT SELECTION AND ENROLMENT

Patients will be identified from:

• Patients with a current or previous hospitalisation for HF

• In-patient or out-patient echocardiography lists

6.1 Identifying participants

Recruitment will take place in cardiology outpatient department and Cardiology wards of Ninewells Hospital. Potentially eligible participants with heart failure will be identified by delegated study staff, who are part of the cardiology team in Ninewells hospital and therefore have a routine access to the records. Permission to recruit patients will be sought from the patients' cardiologists at the start of the study before recruitment. Participants who meet the eligibility criteria (detailed in section 5.11) will be given a patient information sheet (PIS) detailing what the study involves by the clinical team. If the patient agrees to take part (by means of a reply slip), then informed consent will be sought prior to any study-related activities taking place.

6.2 Consenting participants

Once the patient agrees to participate (being given at a minimum 24 hours from receiving the PIS), they will be requested to provide written informed consent by the research team. This will be performed in person in the hospital. Consent will be taken in accordance with good clinical practice (GCP) and in accordance with TASC SOP 07 (Receiving Informed Consent from Potential Participants in Clinical Research). The patient will receive a copy of the signed consent form. Consent will be taken by research team who are delegated to do so and who will have current GCP training.

Where a participant requests to speak with a physician from the study team, the consent process will not be completed until the participant has spoken to the physician and had all their questions answered to their satisfaction and all queries are addresses properly.

Participating in the study is purely voluntary and decision of the participant will not change the management plan of the patient.

If a participant, who has given informed consent, loses capacity to consent during the study, the participant and all identifiable data or tissue collected would be withdrawn from the study. With Participant's permission, any data, which is not identifiable to the research team and is collected prior to withdrawal, will be stored anonymously for analysis purposes.

6.3 Screening for eligibility

All patients with a diagnosis of HF will be eligible to take part in the study. Eligibility will be screened according to inclusion and exclusion criteria. Only one screening assessment is required.

6.4 Ineligible and non-recruited participants

Ineligible patients will be screened out prior to being requested for participation. Details of patients who do not wish to take part will be recorded so they are not approached again in future.

7. DATA COLLECTION AND MANAGEMENT

7.1 Data collection

Clinical Portal and Tayside Echocardiography database, which contain information on inpatient and outpatient care, including demographic information, diagnoses, procedures, prescription records, blood investigation results and echocardiographic parameters will be assessed and analysed. Blood samples will be taken as outlined in section 5.4. Clinical outcomes such as death (including cause), hospitalization (including CV hospitalization and HF hospitalization specifically) will be recorded. We will use a continuation sheet to record participation and visit details.

An Excel spreadsheet will be populated to encompass relevant electronic health record data and in-person study assessments at baseline, day 5 (if applicable) and 6 months as outlined in section 5.4 (Study Assessments).

7.2 Data management system

Data management will be conducted in compliance with TASC SOPs on Data Management, including TASC SOP53 Data Management Systems in Clinical Research. The data management system (DMS) will be Excel as approved by Sponsor. The DMS will be based on the protocol and CRF for the study and individual requirements of the investigators. The CRF will collect only information that is required to meet the aims of the study and to ensure the eligibility and safety of the participant. The study database will be compliant with TASC SOP53 Data Management Systems in Clinical Research and will be manually cross-checked with clinical records to ensure accuracy. The database will be held on a password-protected University computer with access controls and regular updates to operating systems and security software.

The database is managed in line with all applicable principles of medical confidentiality and data laws. The Data Controller will be the University of Dundee and the Data Custodian will be the Chief Investigator.

The CI may delegate CRF completion but is responsible for completeness, plausibility and consistency of the CRF. Any queries will be resolved by the CI or delegated member of the study team.

Database lock will be conducted at the end of the study in compliance with TASC SOP32 Locking Clinical Study Databases.

8. STATISTICS AND DATA ANALYSIS

8.1 Sample size calculation

We have previously found significant between group differences in SIK activity in a 62-patient study[28], so we anticipate that our cohort size should be adequate to identify differences between subgroups of HF patients (e.g. in-patients vs. out-patients). For example, based on this work, n=40 subjects in each group will provide at least 80% power and 5% significance to detect a 20% difference in cytokine analyses between acutely decompensated and chronic stable HF patients.

8.2 Proposed analyses

All data will be analysed using standard statistic software packages such as SPSS or R and performed by the research team. Continuous variables will be expressed as mean ± standard deviation (SD). The differences between normally distributed numeric variables were evaluated by t-test or one-way ANOVA, while non-normally distributed variables will be analysed by Mann-Whitney U test or Kruskal-Wallis variance analysis, as appropriate. Chi-square test will be employed for the comparison of categorical variables. A p value <0.05 will be considered statistically significant. Associations between SIK and inflammatory cytokine levels and echocardiographic parameters and QoL will be measured using linear and logistic regression models. Kaplan- Meier survival curve and Cox regression analysis will be used to assess the association between relevant baseline variables and clinical outcomes.

8.3 Missing data

All possible efforts will be made to ensure that there is no missing data. Methods such as multiple imputation will be used if there is missing data.

8.4 Transfer of data

Data will be anonymised. Where anonymised research data requires to be transferred, an appropriate Data Transfer Agreement will be put in place. However, there are no immediate plans to transfer data, and the important part of this study is the potential to collaborate with other cohorts. The first contact will be made with the Chief Investigator. If summarised, anonymised data is required then this will be provided to the collaborator.

9. STUDY MANAGEMENT AND OVERSIGHT ARRANGEMENTS

9.1 Study management group

The study will be co-ordinated by a Study Management Group (SMG), consisting of e.g. the Chief Investigator (CI) and Principal Investigators (PIs).

9.2 Study steering committee

There will be no specific Study Steering Committee, however, the roles of an SSC will be incorporated within the SMG.

9.3 Data monitoring committee

There will be no specific Data Monitoring Committee, however, the roles of a DMC will be incorporated within the SMG.

9.4 Inspection of records

The CI and all institutions involved in the study will permit study related monitoring, audits, and REC review. The CI agrees to allow the Sponsor or, representatives of the Sponsor, direct access to all study records and source documentation

10. GOOD CLINICAL PRACTICE

10.1 Ethical conduct of the study

The study will be conducted in accordance with the principles of good clinical practice (GCP) and ethical principles outlined in the Declaration of Helsinki. No potentially vulnerable subjects will be enrolled in the study.

In addition to Sponsorship approval, a favourable ethical opinion will be obtained from the appropriate REC and appropriate NHS R&D approval(s) will be obtained prior to commencement of the study.

10.2 Confidentiality and data protection

The CI and study staff will comply with all applicable medical confidentiality and data protection principles and laws with regard to the collection, storage, processing and disclosure of personal data.

The CI and study staff will also adhere to the NHS Scotland Code of Practice on Protecting Participant Confidentiality or equivalent.

All study records and personal data will be managed in a manner designed to maintain participant confidentiality. All records, electronic or paper, will be kept in a secure storage area with access limited to appropriate study staff only. Computers used to collate personal data will have limited access measures via usernames and passwords.

Personal data concerning health will not be released except as necessary for research purposes including monitoring and auditing by the Sponsor, its designee or regulatory authorities providing that suitable and specific measures to safeguard the rights and interests of participants are in place.

The CI and study staff will not disclose or use for any purpose other than performance of the study, any personal data, record, or other unpublished, confidential information disclosed by those individuals for the purpose of the study. Prior written agreement from the Sponsor will be required for the disclosure of any said confidential information to other parties.

Access to collated personal data relating to participants will be restricted to the CI and appropriate delegated study staff.

Where personal data requires to be transferred, an appropriate Data Transfer Agreement will be put in place.

Published results will not contain any personal data that could allow identification of individual participants.

10.3 Insurance and indemnity

The University of Dundee is sponsoring the study.

Insurance – The University of Dundee will obtain and hold a policy of Public Liability Insurance for legal liabilities arising from the study.

Where the study involves University of Dundee staff undertaking clinical research on NHS patients, such staff will hold honorary contracts with Tayside Health Boards which means they will have cover under Tayside's membership of the CNORIS scheme.

Indemnity – The sponsor does not provide study participants with indemnity in relation to participation in the Study but have insurance for legal liability as described above.

11. ADVERSE EVENTS

11.1 Definitions

Adverse Event (AE)	Any untoward medical occurrence in a clinical research participant which does not necessarily have a causal relationship with study participation
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life threatening • requires hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability or incapacity

11.2 Recording and reporting AE

All AEs and/or SAEs will be recorded on the AE Log in the CRF and will be assessed for severity by the CI or delegate. AEs/SAEs will be recorded from the time a participant consents to join the study until the participant's last study visit.

The Investigator will make a clinical judgment as to whether or not an AE is of sufficient severity to require the participant's removal from the study. A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant should, if required, be offered an end of study assessment and be given appropriate care under medical supervision until symptoms cease, or the condition becomes stable. AEs/SAEs will be followed up until 30 days after participant's last visit.

The CI or delegate will ask about the occurrence of AEs/SAEs and hospitalisations at every visit during the study. SAEs which are both unexpected and related to study participation will be submitted on an HRA NCTIMP Safety Report form to the REC by the CI, within 15 days of becoming aware of the SAE, and copied to the Sponsor Research Governance Office.

Worsening of the condition under study will not be classed as an AE but will be defined as an outcome. Pre-specified outcome(s) will not be classed as an AE but as an outcome. Elective admissions and hospitalisations for treatment planned prior to randomisation, where appropriate, will not be considered as an AE. However, AEs/SAEs occurring during such hospitalisations will be recorded.

12. ANNUAL REPORTING REQUIREMENTS

Annual reporting will be conducted in compliance with TASC SOP 15: Preparing and Submitting Progress and Safety Reports in CTIMPs and Non-CTIMPs, as a condition of sponsorship and as a condition of a favourable opinion from a REC. An HRA Annual Progress Report for NCTIMPs will be prepared and submitted by the CI to REC, and copied to the Sponsor, on the anniversary date of the REC favourable opinion.

Any safety reports additional to SAE reports, for example, reports of a DMC, will be sent by the CI to REC, with a Safety Report Form, and to the Sponsor.

13. STUDY CONDUCT RESPONSIBILITIES

13.1 Protocol amendments, deviations and breaches

The CI will seek approval for any amendments to the Protocol or other study documents from the Sponsor, REC (if substantial) and NHS Tayside R&D Office. All amendments will be reviewed and approved prior to implementation as per Sponsor SOPs. all breaches will be reported to the Sponsor as per Sponsor SOP.

13.2 Study record retention

Archiving of study documents will be for five years after the end of study.

13.3 End of study

The end of study is defined as 6 months after the last patient last visit (LPLV).

The CI will ensure that any appropriate follow up is arranged for all participants.

A summary report of the study will be provided to the Sponsor and REC within 1 year of the end of the study.

14. REPORTING, PUBLICATIONS, AND NOTIFICATION OF RESULTS

14.1 Authorship policy

Ownership of the data arising from this study resides with the study team and their respective employers. On completion of the study, the study data will be analysed and tabulated, and a clinical study report will be prepared.

14.2 Publication

The clinical study report will be used for publication and presentation at scientific meetings. Investigators have the right to publish orally or in writing the results of the study.

Summaries of results will also be made available to Investigators for dissemination within their clinical areas (where appropriate and according to their discretion).

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