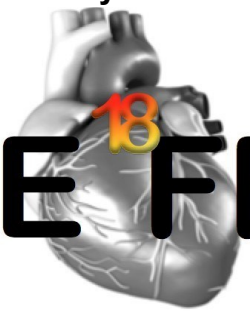
Academic and Clinical Central Office for Research and Development

Study Protocol

The PREFFIR Study



Prediction of Recurrent Events with ^{18}F-Fluoride to Identify Ruptured and High-risk Coronary Artery Plaques in Patients with Myocardial Infarction

Co-sponsors	University of Edinburgh & NHS Lothian ACCORD The Queen's Medical Research Institute 47 Little France Crescent Edinburgh EH16 4TJ
Funder	Wellcome Trust
Funding Reference Number	WT103782AIA - Senior Investigator Award
Chief Investigator	Professor David Newby
REC Number	15-SS-0059
EudraCT Number	2014-004021-41
ClinicalTrials.gov ID number	NCT02278211
Number and Date	Version 11.0 - 24 February 2022

COORDINATING CENTRE

Chief Investigator	**Co Investigator**
Professor David E Newby Centre of Cardiovascular Science Chancellor's Building 51 Little France Crescent Edinburgh EH16 4SB Tel: 0131 242 6515 Fax: 0131 242 6379 Email: d.e.newby@ed.ac.uk	Dr Marc Dweck Centre of Cardiovascular Science Chancellor's Building 51 Little France Crescent Edinburgh EH16 4SB Tel: 0131 242 6364 Email: marc.dweck@nhslothian.scot.nhs.uk
Clinical Research Fellow **Dr Marwa Daghem** Centre of Cardiovascular Science Chancellor's Building 51 Little France Crescent Edinburgh EH16 4SB Tel: 07500931090 Email: mdaghem@exseed.ed.ac.uk	**Co-sponsor Representative** Dr Fiach O'Mahony University of Edinburgh Queen's Medical Research Institute 47 Little France Crescent Edinburgh EH16 4SB Tel: 0131 242 6226 Fax: 0131 242 9418 Email: Fiach.O'Mahony@ed.ac.uk
Trial Manager Claire Battison Edinburgh Clinical Trials Unit University of Edinburgh NINE, 9 Little France Road Edinburgh EH16 4UX Tel: 0131 651 9919 Email: c.battison@ed.ac.uk	**Trial Statistician** Professor Steff Lewis Edinburgh Clinical Trials Unit University of Edinburgh NINE, 9 Little France Road Edinburgh EH16 4UX Tel: 0131 651 9956 Email: steff.lewis@ed.ac.uk

CONTENTS

PROTOCOL APPROVAL

PRE[18]FFIR Study

Signatures

Professor David Newby

Chief Investigator	Signature	Date

Professor Steff Lewis

Study Statistician	Signature	Date

Dr.Fiach O'Mahony

Sponsor(s) Representative	Signature	Date

Principal Investigator	Signature	Date

LIST OF ABBREVIATIONS

ACCORD	Academic and Clinical Central Office for Research & Development - Joint office for University of Edinburgh and NHS Lothian
AE	Adverse Event
AR	Adverse Reaction
CI	Chief Investigator
CT	Computer Tomography
CTA	Clinical Trial Authorisation
CTCA	Computer Tomography Coronary Angiography
CTIMP	Clinical Trial of an Investigational Medicinal Product
CRF	Case Report Form
DNA	Deoxyribose Nucleic Acid
eGFR	Estimated Glomerular Filtration Rate
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
ISF	Investigator Site File
IVUS	Intravascular Ultrasound
MHRA	Medicines and Healthcare products Regulatory Authority
NIMP	Non Investigational Medicinal Product
NIRS	Near Infrared Spectroscopy
OCT	Optical Coherence Tomography
PET	Positron Emission Tomography
R&D	Research & Development
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
UAR	Unexpected Adverse Reaction

1 INTRODUCTION

1.1 BACKGROUND

1.1.1 Pathogenesis of Coronary Artery Disease

Coronary plaque rupture of mildly stenotic coronary atherosclerosis is the commonest cause of acute coronary thrombosis, myocardial infarction and death [Davies, 2000]. It is closely linked to hypercholesterolaemia and has a dominant inflammatory phenotype. Classical histological features include a lipid-rich pool, thin fibrous cap, paucity of vascular smooth muscle cells and an intense inflammatory cell infiltrate [Davies, 2000].

Plaque rupture does not invariably lead to thrombotic occlusion of the coronary artery. Coronary plaque events are very common, and the majority of such events do not cause coronary occlusion [Mann & Davies, 1999; Davies, 2000]. Here, plaque rupture and thrombosis are organised, remodelled and incorporated into the atherosclerotic plaque itself. Indeed, 80% of plaques that cause over 50% diameter stenosis have evidence of old healed plaque rupture with incorporation of thrombus into the atheroma [Mann & Davies, 1999]. This process contributes to the progression of coronary atherosclerosis and explains the first appearance, or step change in severity, of angina pectoris.

Coronary plaque events are often not isolated. Plaque rupture can occur throughout the body and is not confined to a single vascular bed. Moreover, in patients with acute coronary syndromes, it is common for multiple plaque events to occur simultaneously and beyond the culprit lesion itself. Post-mortem studies indicate that, on average, 2.4 coronary thrombotic events occur in each patient who presents with fatal coronary heart disease [Mann & Davies, 1999; Davies, 2000]. This suggests that systemic and generalised mechanisms and mediators play an important role in addition to local factors within the coronary artery wall.

In summary, coronary atherosclerosis is characterised by multiple and recurrent plaque rupture events that are often sub-clinical and cause step wise growth of the plaque as it heals and remodels. Patients undergoing repeated plaque rupture events are likely to be at increased risk of myocardial infarction.

1.1.2 Non-invasive Imaging of the Vulnerable Plaque

In the study of coronary artery disease, many researchers have searched for a non-invasive imaging biomarker of plaque vulnerability and rupture. For the first time, we have demonstrated that 18F-fluoride positron emission tomography can detect high-risk coronary plaque in patients with stable coronary artery disease [Joshi *et al*, 2014].

In the vasculature, 18F-fluoride acts as a marker of novel calcification activity [Dweck *et al*, 2012a; Dweck *et al*, 2013]. Similar to other conditions, calcification in coronary atheroma occurs as a healing response to intense necrotic inflammation, making 18F-fluoride a useful marker of high-risk atherosclerotic plaque. We have previously demonstrated increased uptake of this tracer in the coronary vasculature localizing to individual coronary lesions and identifying patients with increased cardiovascular risk factor profiles [Dweck *et al*, 2012b].

More recently we have conducted a prospective study of 40 patients with myocardial infarction in whom 18F-fluoride localised to the culprit plaque (Figure 1) in over 90% of patients [Joshi *et al*, 2014]. This finding was confirmed in 12 patients with a recent stroke undergoing carotid endartectomy where 18Ffluoride uptake was observed at the site of plaque rupture in 100% of patients and this uptake correlated with increased calcification activity and areas of necrosis on histology. Finally, we studied 40 patients with stable coronary artery disease. Increased uptake was observed in 45% of these patients and this again localised to individual coronary plaques (Figure 2). Interestingly these lesions were associated with multiple high-risk markers on radiofrequency and gray-scale intravascular ultrasound (necrotic core, positive remodeling and microcalcification). Importantly, plasma high-sensitivity troponin concentrations were much higher in patients with 18F-fluoride positive plaques compared to patients without evidence of uptake (7.89±9.34 *versus* 3.10±1.89 ng/L, P=0·047). The latter observation is of particular interest as it supports the hypothesis that 18F-fluoride is detecting subclinical plaque rupture in those with stable disease, similar to its mechanism of activity following myocardial infarction. Moreover, plasma troponin concentrations measured by a highsensitivity assay also predict an adverse outcome amongst patients with stable coronary artery disease [Omland *et al*, 2013] and provide a useful surrogate biomarker of therapeutic efficacy.

1.1.3 Recurrent Events Following Acute Myocardial Infarction

Myocardial infarction and stroke are two of the commonest causes of hospitalisation, major disability and death in the United Kingdom. However, despite many billions of pounds being spent each year on their treatment, recurrent cardiovascular events are common. Indeed, within one year of acute myocardial infarction, one in five patients have recurrent events despite optimal medical therapy [Mills et al, 2011], and one half of these recurrent events occur at sites not felt to have caused their initial presentation [Stone et al, 2011]. Coronary calcification is pathognomonic of atherosclerosis and recent studies in Egyptian mummies have demonstrated that it has been present in humans for millennia [Thompson et al, 2013]. Despite this, the mechanisms of its formation have yet to be precisely defined. Furthermore, the nature and extent of vascular calcification may vary according to the pathophysiological setting and underlying disease process. Given that it is independently associated with a 3 to 4-fold increased risk of death [Detrano et al, 2008], a better understanding of the pathophysiology and mechanisms of vascular calcification will be critical to our understanding of cardiovascular disease. This is particularly important if we are to determine its role in the initiation, progression and consequences of atherosclerosis. This may provide novel insights into the pathophysiology of atherosclerosis as well as identify new and as yet unexplored pathways that could be the target for future life-saving therapeutic interventions. For example, although highly successful in treating cardiovascular disease, statins only prevent 25% of recurrent cardiovascular events, and have no effect on the progression of vascular calcification. The majority of recurrent events therefore

remain unchecked and we urgently need more effective and comprehensive interventions.
Non-invasive imaging techniques to detect early active vascular calcification in atherosclerosis have the potential to identify high-risk and ruptured atherosclerotic plaques, thereby providing a major novel biomarker of plaque vulnerability. This will provide a platform to explore and follow the *in vivo* pathogenesis of atherosclerosis, as well as assess novel anti-atherosclerotic interventions. This will also facilitate the more accurate identification of culprit atherosclerotic plaque in the precipitation of myocardial infarction and stroke, allowing better delivery and targeting of our therapies at both the lesion and patient level. This approach would potentially be a major advance for the treatment of unstable atherosclerotic disease.

1.1.4 Stratified Medicine and Intervention

Current treatment decisions for patients with acute myocardial infarction are based on a large number of major multi-centre international randomised controlled trials. These trials demonstrate major secondary preventative benefits with the use of anti-platelet therapy, statins, angiotensin-converting enzyme inhibition and beta-blockade following acute myocardial infarction. However, all patients are treated with the same combination of medications with little attempt to risk stratify patients and select those who have the most to benefit from such therapies.
Emergency or immediate coronary angiography with a view to coronary revascularisation is now recommended in the vast majority of patients presenting with acute myocardial infarction. Indeed, 80% of patients admitted with non-ST segment elevation myocardial infarction in the United Kingdom undergo coronary angiography with a view to coronary revascularisation during their index hospital admission [MINAP, 2013]. However, coronary revascularisation following myocardial infarction focuses on treating flowlimiting stenoses (≥70% luminal stenosis) with coronary revascularisation strategies such as percutaneous coronary intervention and coronary artery bypass graft surgery. However, we know that the majority of plaque rupture events occur on non-flow limiting stenoses (see section 1.1), and this observation questions whether we are over treating a large number of patients and indeed coronary artery lesions without actually tackling the future potential culprit lesions. Using 18F-fluoride, we have "the opportunity to better assess the commonly accepted belief that most myocardial infarctions are caused by rupture of previously non-obstructive plaques" [Thomas & Haraszti, 2014].

1.1.5 Coronary Microcalcification Activity

In preliminary proof-of-concept studies, we have assessed a global measure of coronary 18F-fluoride uptake in patients with coronary artery disease. We have termed this measure 'coronary microcalcification activity' and is analogous to the computed tomography-derived Agatston coronary artery calcium score. We have shown that total coronary microcalcification activity can be measured in a reproducible manner [Tzolos et al, 2020], and that it correlates with measures of high-risk low-attenuation plaque [Kwiecinski et al, 2020a]. In a post-hoc

analysis of nearly 300 patients with coronary artery disease from 2 centres, coronary microcalcification activity was the strongest predictor of fatal or nonfatal myocardial infarction over 42 months of follow up [Kwiecinski et al, 2020b]. It outperformed all standard cardiovascular risk factors, risk scores and standard measures of coronary artery disease severity.

1.2 RESEARCH HYPOTHESIS

The central hypothesis of the PRE18FFIR study is that coronary 18F-fluoride uptake can identify high-risk and ruptured atherosclerotic plaque in patients with recent myocardial infarction. This has the potential to assist in the diagnosis, risk stratification, investigation, management and treatment of patients with acute myocardial infarction.

1.3 RATIONALE FOR THE STUDY

We have investigated and described the use of 18F-fluoride positron emission tomography and computed tomography coronary angiography to identify highrisk or ruptured coronary atherosclerotic plaque [Joshi et al. Lancet 2014]. This is the first non-invasive imaging technique to achieve this and it has been heralded as a major advance with wide reaching ramifications for the diagnosis, investigation, and treatment of coronary artery disease [Thomas & Haraszti, 2014]. Importantly, it may provide the basis of a new paradigm in the treatment and management of patients with myocardial infarction by tailoring therapy to the presence and extent of plaque inflammation and rupture rather than the anatomical severity of luminal stenoses. More targeted and focused investigations and interventions have the potential to shorten hospital stay, to avoid the indiscriminate application of expensive therapies, and to focus and to maximise the impact of our current successful therapies to reduce recurrent major adverse cardiac events.

The study rationale is therefore to confirm our preliminary findings in a broad range of patients across different centres throughout the United Kingdom and across the world. Specifically, the study is intended to confirm whether coronary 18F-fluoride uptake identifies high-risk or ruptured coronary atherosclerotic plaque, and to determine if coronary 18F-fluoride uptake is predictive of disease progression and clinical outcome. We have also planned two sub-studies to assess the reproducibility and natural history of 18F-fluoride uptake and an additional sub-study to provide correlation with in vivo plaque characterisation tests. These sub-studies are described in more detail in separate protocols.

2 STUDY OBJECTIVES

2.1 OBJECTIVES

2.1.1 Primary Objective

- To determine whether coronary 18F-fluoride uptake is associated with major adverse cardiac events in patients with multi-vessel coronary artery disease and recent myocardial infarction.

2.1.2 Secondary Objectives

In patients with multi-vessel coronary artery disease and recent myocardial infarction, to determine whether coronary ^{18}F-fluoride uptake:

- Is associated with secondary clinical outcomes including all-cause death, cardiac death, non-fatal recurrent myocardial infarction, and, unscheduled coronary revascularisation..
- Can identify areas of high-risk or ruptured atherosclerotic plaque in a large broad population of patients across multiple sites.
- Can predict the territory of subsequent myocardial reinfarction.
- Can identify areas of future progression of coronary atherosclerotic plaque (volume and calcification)

2.1.3 Safety Objectives

- To determine the radiation exposure and effective dose associated with 18F-fluoride positron emission tomography and computed tomography coronary angiography.
- To identify adverse events associated with 18F-fluoride positron emission tomography and computed tomography coronary angiography.

2.1.4 Exploratory Objectives

We will initially explore whether the identification of 18F-fluoride positive coronary atherosclerotic plaque has the potential to guide patient management. Here positron emission tomography and computed tomography coronary angiography results will be compared with the subsequent application of coronary revascularisation and optimal medical therapy. Importantly, we will also explore the feasibility of using the identification of 18F-fluoride positive coronary atherosclerotic plaque to guide coronary revascularisation and intervention. For example, how many stenotic but 18F-fluoride negative lesions undergo intracoronary stenting, and how many 18F-fluoride positive lesions are left untreated and are responsible for recurrent cardiovascular events?

2.2 ENDPOINTS

2.2.1 Primary Endpoints

- Cardiac death, non-fatal recurrent myocardial infarction, or unscheduled coronary revascularisation.

2.2.2 Secondary Endpoints

These will include:

- All-cause death
- Cardiac death
- Non-fatal recurrent myocardial infarction
- Unscheduled coronary revascularisation

- 18F-NaF uptake localisation to culprit plaque causing the index myocardial infarction
- Territory of subsequent myocardial reinfarction.
- Coronary artery plaque progression by computed tomography coronary angiography. Plaque volume, composition and calcification.

2.2.3 Safety Endpoints

- Dose-length product and effective radiation dose associated with PET and CT scans.
- Adverse events experienced during the scan or self-reported within 48 hours of PET and CT scans.

3 STUDY DESIGN

This is a multicentre study of an investigational medical product with longitudinal follow-up for disease progression and clinical outcomes.

3.1 RATIONALE FOR STUDY DESIGN

We wish to confirm our preliminary findings in a larger multicentre study of patients with recent myocardial infarction. We will undertake a study in patients with recent myocardial infarction who will undergo coronary 18F-fluoride positron emission tomography and computed tomography coronary angiography immediately following hospitalisation. We will conduct a longitudinal cohort follow-up of participants who will undergo clinical follow-up of at least 2 years duration when repeat computed tomography coronary angiography will be performed.

Before conducting a randomised controlled trial using coronary 18F-fluoride uptake to stratify and guide treatment, it is imperative that we confirm the diagnostic and prognostic importance of coronary 18F-fluoride uptake within a large multicentre study.

4 STUDY POPULATION

4.1 NUMBER OF PARTICIPANTS

We will recruit approximately 700 patients with recent myocardial infarction and multi-vessel coronary heart disease.**4.2 INCLUSION CRITERIA**

For inclusion in the study subjects will fulfil the following criteria:

1. Patients aged ≥50 years with recent (<21 days) type 1 myocardial infarction and angiographically proven multi-vessel coronary artery disease defined as at least two major epicardial vessels with any combination of either (a) >50% luminal stenosis, or (b) previous

revascularisation (percutaneous coronary intervention or coronary artery bypass graft surgery).

2. Provision of informed consent prior to any study specific procedures

4.3 EXCLUSION CRITERIA

Subjects will not enter the study if any of the following exclusion criteria are fulfilled:

1. Inability or unwilling to give informed consent
2. Women who are pregnant, breastfeeding or of child-bearing potential (women who have experienced menarche, are pre-menopausal and have not been sterilised) will not be enrolled into the trial
3. Major intercurrent illness with life expectancy <2 year
4. Renal dysfunction (estimated glomerular filtration rate ≤30 mL/min/1.73 m^2)
5. Contraindication to iodinated contrast agent, positron emission tomography or computed tomography
6. Permanent or persistent atrial fibrillation
7. Previous screening as part of the trial

4.4 CO-ENROLMENT

Co-enrolment with a non-CTIMP or another CTIMP clinical trial may be allowed provided this is not expected to place an undue burden upon participants and their families and will not compromise the primary end point of either trial. Consideration will also be given to the total exposure to ionising radiation should additional studies require further exposure.

Co-enrolment with another CTIMP will only be permitted with agreement of the Sponsors, Trial Steering Committees, and Chief Investigators of both studies.

5 PARTICIPANT SELECTION AND ENROLMENT

5.1 IDENTIFYING PARTICIPANTS

Patients presenting with myocardial infarction will be approached for study recruitment by their usual care team. This will normally be undertaken during their index hospital admission. Patients will be provided with a Patient Information Sheet and given an opportunity to ask questions about participation in the trial.

5.2 CONSENTING PARTICIPANTS

After an appropriate length of time, patients willing to participate in the study will be asked to consent and undergo screening. Written informed consent will be

obtained by a suitably qualified member of the research team before any study related procedures are performed.

5.3 SCREENING FOR ELIGIBILITY

Screening for eligibility based on pre-existing clinical investigations (i.e. standard clinical biochemical and haematological investigations and coronary angiographic findings) will be performed by a member of the research team and will only take place once written consent has been obtained. Where it is not possible for participants to be consented during their initial hospital admission, they may be asked to attend an additional study visit during which consent will be taken and eligibility confirmed. Once a patient has agreed to participate and is deemed eligible, they will be invited to attend the baseline visit for 18Ffluoride positron emission tomography and computed tomography coronary angiogram scans.

5.4 INELIGIBLE AND NON-RECRUITED PARTICIPANTS

Ineligible and non-recruited patients will receive standard medical care. An anonymised log will be kept of patients who were screened for the study and subsequently found to be ineligible or not recruited.

5.5 PROCEDURES FOR HANDLING PARTICIPANTS INCORRECTLY ENROLLED

Patients identified as being incorrectly enrolled prior to their initial CT-PET scan will be excluded from the primary study analysis and replaced. Patients who are identified as incorrectly enrolled but have undergone their initial CT-PET will be included in the primary analysis and we will endeavour to continue to collect study data without involving the participant in any further study related procedures.

5.6 WITHDRAWAL OF STUDY PARTICIPANTS

Participants are free to withdraw from the study at any point or a participant can be withdrawn by the investigator. If withdrawal occurs, the primary reason for withdrawal will be documented in the participant's case record form, if possible. The patient will have the option of withdrawal from (i) all or specific further study procedures but continued collection of routine clinical and safety data, (ii) all further aspects of the study with no further collection of any data, and (iii) all aspects of the trial with removal of all previously collected data.

6 INVESTIGATIONAL MEDICINAL PRODUCT

6.1 STUDY DRUG

Investigational product	Dosage form and strength	Manufacturer
[18F] Sodium Fluoride	250 MBq	See 6.1.2

6.1.1 Study Drug Identification

[18F] Sodium Fluoride (18F-NaF) sterile solution for injection

6.1.2 Study Drug Manufacturers

Manufacturing will be performed by:

Clinical Research Imaging Centre (CRIC)
University of Edinburgh
47 Little France Crescent
Edinburgh
EH16 4TJ
Licence: MIA (IMP) 1384

PETNET Solutions
Lesley Harrison Building
Mount Vernon Hospital
Northwood
Middlesex
HA6 2RN
Licence: MIA (IMP) 21750

PETNET Solutions
Nottingham City Hospital
Gate 1
Hucknall Road
Nottingham
NG5 1PB
Licence: MIA (IMP) 21750

6.1.3 Marketing Authorisation Holder

There is currently no marketing authorisation for [18F] Sodium Fluoride (18F-NaF)

6.1.4 Labelling and Packaging

Labelling and packaging of the [18F] Sodium Fluoride (18F-NaF) will be carried out by:

Clinical Research Imaging Centre (CRIC)

University of Edinburgh
47 Little France Crescent
Edinburgh
EH16 4TJ
Licence: MIA (IMP) 1384

PETNET Solutions
Lesley Harrison Building
Mount Vernon Hospital
Northwood
Middlesex
HA6 2RN
Licence: MIA (IMP) 21750

PETNET Solutions
Nottingham City Hospital
Gate 1
Hucknall Road
Nottingham
NG5 1PB
Licence: MIA (IMP) 21750

6.2 DOSING REGIME

Single intravenous injection

6.3 OVERDOSE

Given that the 18F-fluoride will be administered as a single intravenous injection by trained personnel, accidental or unintentional overdose is not anticipated to occur. Moreover, the doses and quantities of sodium fluoride are very low (<430 µg) and >1,000-fold below the dose (3-5 mg/kg) where toxicity symptoms may appear and >1,000,000-fold below the anticipated lethal dose (5-10 g). However, if an overdose of sodium fluoride were to occur then radiation exposure and sodium fluoride toxicity will be the primary considerations. Excretion should be accelerated in order to reduce the dose taken by the patient. For this reason, forced diuresis (furosemide and fluid) should be applied to the patient.

For radiation overexposure, the patient will be contained within a safe environment to allow radioactive decay to occur to acceptable levels. Monitoring for the consequences of excess radiation exposure (such as bone marrow toxicity) will be undertaken as required. Symptoms of sodium fluoride toxicity include abdominal pain, abnormal taste (salty or soapy taste), convulsions, diarrhoea, drooling, headache, arrhythmia, nausea, hypopnea, bradycardia, tremors, vomiting, and weakness. For an intravenous overdose, there are no specific antidotes and supportive measures will be undertaken as clinically

indicated, such as hydration and anti-emetic therapy. Consideration will be given to intravenous calcium supplementation to compete with the excess fluoride ions especially in the presence of problematic arrhythmias.

6.4 OTHER MEDICATIONS

6.4.1 Non-Investigational Medicinal Products

At the time of positron emission tomography and computed tomography coronary angiography, patients may receive oral and/or intravenous betablockade, such as metoprolol 5-100 mg, to slow the heart rate to below 65 beats per minute to maximise image quality and reduce radiation exposure. Glyceryl trinitrate spray or tablet will be administered sublingually (200-400 µg) to induce coronary vasodilatation to enhance image quality of the coronary angiogram.

6.4.2 Permitted Medications

As per the standard of care for treatment of myocardial infarction, unless contraindicated, all patients will be encouraged to be maintained on aspirin 75 mg once daily and maximally tolerated doses of statin, angiotensin-converting enzyme inhibition and beta-blocker therapy as clinically indicated and in accordance with local guidelines. Patients will be encouraged to be maintained on a P2Y12 receptor antagonist for at least 1 year.

There are no study specific prohibited medications.

7 STUDY ASSESSMENTS

Trial participants will undergo 3 or 4 study visits (Table 1). During their initial hospital admission, medical notes will be screened prior to consent and potentially eligible patients will be approached to take part in the study. Those patients who are potentially eligible and wish to participate will be consented and routine clinical data will be collected following consent (screening visit). Where it is not possible for potential participants to be consented during their initial hospital admission, they may be asked to attend an additional study visit during which consent will be taken and eligibility confirmed. The participants will then attend the baseline visit where they will have a 18F-fluoride positron emission tomography, computed tomography (CT) calcium scan and CT coronary angiography (CTCA) scan performed before attending for a final clinical review and 2-year computed tomography calcium scan and CT coronary angiogram (2-year visit) when further routine clinical data will be collected. In order to assess for clinical cardiac events participants will contacted by telephone 1 year after their baseline PET and CTCA scans were performed. Following the 2-year visit, data on study outcomes will continue to be collected until study completion (last patient, last visit) via periodic review of the electronic health record and additional annual telephone review as required. This will ensure that late study outcomes can be evaluated over the duration of the study.

In order to determine long-term outcomes, we may continue to monitor clinical events via annual review of patient electronic clinical records for a period of 5 years following the completion of the participant's final study visit.

Table 1: Study Assessments

	Screening Visit 1 to 30 days prior to baseline visit (During either initial hospital admission or additional study visit)	**Baseline Visit** (Day 0)*	Additional Visits for Reproducibility and Natural History Sub studies only				**1 Year Review** (-2 weeks to +26 weeks)	**2-Year Visit**** (0 to + 52 weeks)
			Week 2 (±1 week)	Week 12 (±2 weeks)	Week 26 (±2 weeks)	Week 52 (±2 weeks)		
Eligibility Criteria and PIS	X							
Consent	X							
Concomitant medications	X							
Clinical Assessment	X							
Haematology, biochemistry and Cardiac Enzymes	X	This data will be collected from the existing patient record and should not require additional procedures						
ECG	X							
GRACE Score Calculation	X							
AE/SAE Reporting		X						X
18F-Fluoride PET		X	X	X	X	X		
CT attenuation correction		X	X	X	X	X		
CT Calcium Scan		X						X
CT Coronary Angiogram		X	X	X	X	X		X
			Additional PET and CT scans will be performed in those patients involved in the sub studies only					
Telephone Review							X	
		Additional annual telephone follow up with review of electronic health records if warranted until study completion (last patient, last visit)						

*If it is not possible for the baseline PET-CT scan to be performed within 30 days of the screening visit, due to either the scanner becoming unavailable or a problem with the 18F-NaF manufacture, then the scan will be performed within 90 days of the screening visit.

**Where it is not possible for the CTCA scan at the 2 year visit to be performed at the same time as the other 2 year visit assessments, the CTCA scan may be performed on a separate date.

7.1 SAFETY ASSESSMENTS

The dose-length product and effective radiation dose will be recorded for all study scans: baseline 18F-fluoride positron emission tomography and computed tomography coronary angiography scan, and the final 2-year computed tomography coronary angiogram.

7.2 STUDY ASSESSMENTS

This is a prospective multi-centre observational cohort study. Subjects will attend for clinical assessments at the screening visit (review of clinical records and confirmation of eligibility), baseline visit (18F-fluoride positron emission tomography, computed tomography calcium scan and CT coronary angiography scan), and at 2 years (repeat computed tomography calcium scan and CT coronary angiogram). Clinical outcome data will be obtained from routinely collected clinical data in the electronic health record and, where appropriate, annual telephone calls to the patients or General Practitioners.

7.2.1 Screening Assessment

Screening visit assessments will include clinical history, review of patient records to confirm study eligibility and record clinical profile, standard clinical biochemical and haematological variables, 12-lead electrocardiogram, the Global Registry of Acute Coronary Events (GRACE) score and the invasive coronary angiogram findings and percutaneous coronary interventional procedures.

7.2.2 Baseline Assessment

Baseline visit assessments will include 18F-fluoride positron emission tomography, computed tomography calcium scan and CT coronary angiography scan. AE/SAE’s will also be recorded.

7.2.3 Follow Up Assessments

Participants will be contacted by telephone at 12 months and periodically thereafter until the last recruited patient has completed their 2-year follow-up visit. The purpose of the telephone interview is to review any clinical cardiovascular events that may have occurred in the intervening period. They will attend for a final study visit 2 years after their initial CT-PET scans. At the final study visit, patients will undergo a repeat computed tomography calcium scan and CT coronary angiogram. On-going periodic review of the electronic health records will continue throughout the study and may continue for a period of 5 years after study completion in order to assess long-term outcomes. Additional funding and approval will be sought if this is to continue after study completion.

7.2.4 Positron Emission and Computed Tomography Coronary Angiography

Patients may receive oral and/or intravenous beta-blockade, such as metoprolol 5-100 mg, to slow the heart rate to below 65 beats per minute to maximise image quality and reduce radiation exposure. Glyceryl trintrate spray or tablet will be administered sublingually (200-400 µg) to induce coronary vasodilatation to enhance image quality of the coronary angiogram.
All patients will undergo dual cardiac and respiratory-gated positron emission and computed tomography imaging of the coronary arteries. Study subjects will be administered a target dose of 250 MBq 18F-fluoride intravenously and subsequently rested in a quiet environment for 60 min. An attenuation correction computed tomography scan will then be performed, followed by positron emission tomography imaging of the thorax in list-mode for 30 min. Computed tomography coronary calcium scan and angiography will be undertaken in the same visit as the 18F-fluoride scan and again at 2 years. An electrocardiogram-gated breath-hold computed tomography scan of the coronary arteries will be performed. A bolus of contrast (BMI adjusted) will be injected intravenously after determining the appropriate trigger delay with a test bolus. Because the use of contrast is required for the CT scan study participants may require pre-procedural check of estimated glomerular filtration rate (eGFR) according to local study site imaging protocols. If, however, a participant develops a contraindication to the contrast agent prior to the 2-year computed tomography calcium scan, the computed tomography calcium scan can be performed without contrast.

The positron emission tomography scans will be reconstructed in multiple phases of the cardiac cycle, with the diastolic phase (50-75%) used for analysis. Additional reconstructions will be undertaken as necessary. Positron emission tomography scans will correct for cardiac motion correction using electrocardiogram-gated images.

Further technical details of the specific CT-PET scanning procedures and how inter-site imaging standardisation will be achieved will be described in a separate scanning document.

7.3 SUB-STUDY ASSESSMENTS

There will be three main sub-studies in relation to coronary 18F-fluoride uptake that will assess (i) short-term reproducibility, (ii) time course, and (iii) *in vivo* plaque characterisation. These will be performed only at the lead centre in Edinburgh and are described in more detail in separate protocols. Any participant will only be involved in one sub-study that requires a repeat 18Ffluoride positron emission tomography and computed tomography coronary angiogram in addition to the main PRE^{18}FFIR study protocol.

7.3.1 Short-term Reproducibility (PRE^{18}FFIR-REPRO)

Following the baseline assessments, a repeat 18F-fluoride positron emission tomography and computed tomography coronary angiogram will be performed within 21 days of the initial scan. This will allow the assessment of scan-rescan reproducibility. This will be performed in 20 patients.

7.3.2 Time Course (PRE^{18}FFIR-TIME)

Following the baseline assessments, a repeat 18F-fluoride positron emission tomography and computed tomography coronary angiogram will be performed within, 12±2, 26±2 and 52±2 weeks of the initial scan. This will allow a description of the natural history of 18F-fluoride uptake and positivity. This will be performed in three cohorts of 20 patients each. Any single patient will only undergo a maximum of two 18F-fluoride positron emission tomography and three computed tomography coronary angiography scans.

7.3.3 *In Vivo* Plaque Characterisation (PRE^{18}FFIR-IMAGE)

In 80 patients, intravascular ultrasound, near infra-red spectroscopy and optical coherence tomography will be performed on the culprit and control non-culprit lesions at the time of invasive coronary angiography. This will permit detailed characterisation of plaque composition and structure with which to compare the 18F-fluoride positron emission tomography and computed tomography coronary angiography scans.

8 DATA COLLECTION

All trial data will be recorded onto paper case record forms (CRF) by a member of the research team and then entered into an electronic database designed and developed by the Edinburgh Clinical Trials Unit.

9 DATA MANAGEMENT

9.1.1 Personal Data

The following personal data will be collected as part of the research:

- Participant’s name
- Address and contact details
- Date of birth
- GP contact details
- Community Health Index (CHI) number or National Health Service (NHS) number.

9.1.2 Data Information Flow

The participant's name, address and contact details will be used by the research teams at the sites to contact their patients to arrange study visits. The GP contact details will be used by the research staff at sites to send a letter to the participant's GP advising them their patient is taking part in the trial. The participant's date of birth and CHI/NHS number will be collected and used to perform record linkage with national health registries to assess the long-term outcomes of heart artery inflammation. All personal data will be stored securely for a minimum of 5 years after the study has finished.

9.1.3 Transfer of Data

Data collected or generated by the study may be transferred to external individuals or organisations outside of the Sponsoring organisation(s). It may be provided to researchers running other research studies outwith NHS Lothian/University of Edinburgh. Following publication of the primary paper, a de-identified individual participant data set will be submitted to a data archive for sharing purposes. Participant information will only be used by organisations and researchers to conduct research in accordance with the UK Policy Framework for Health and Social Care Research**.** Where this information includes identifiable information, it will be held securely with strict arrangements about who can access the information.
In order to perform data linkage participant's personal details including their CHI number or NHS number will be transferred to national health registries, central NHS bodies and NHS boards.

9.1.4 Data Controller

A data controller is an organisation that determines the purposes for which, and the manner in which, any personal data are processed.

The University of Edinburgh and NHS Lothian are joint data controllers along with any other entities involved in delivering the study that may be a data controller in accordance with applicable laws (e.g. the site)

9.1.5 Data Breaches

Any data breaches will be reported to the University of Edinburgh and NHS Lothian Data Protection Officers who will onward report to the relevant authority according to the appropriate timelines if required.

10 STATISTICS AND DATA ANALYSIS

10.1 SAMPLE SIZE CALCULATION

Based on the assumption that one third of patients will have multiple 18Ffluoride positive plaques, and that the proportion of patients who have died or had a recurrent myocardial infarction by one year will be 30% in patients with multiple 18F-fluoride positive plaques, and 20% in other patients, we need 692 (231+461) subjects (power=80%, p= 2 sided 0.05). This proportion of events is

conservative, as we observed 20% deaths or recurrent myocardial infarctions at 1-year in consecutive unselected patients hospitalised with myocardial infarction [Mills et al, 2011; Mills et al, 2012]. In our preliminary data [Joshi et al, 2014], 25-45% of patients had untreated 18F-fluoride positive plaques. This calculation is from a continuity corrected chi-squared test. As the analysis will be time-to-event, we would expect to need 10% fewer patients, and therefore this calculation allows for 10% missing data.

10.2 PROPOSED ANALYSES

10.2.1 Description of Analysis Sets

The primary analyses will be based on all patients who have undergone the initial CT-PET scan.

10.2.2 Methods of Statistical Analysis

The primary analysis will be performed by calculating Kaplan-Meier estimates of the 'survival' curves for the time to first event (cardiac death, recurrent myocardial infarction, or unscheduled coronary revascularisation) during follow up, and comparing these estimates for patients with and without coronary microcalcification activity using a log-rank test. The results will be expressed as the estimated hazard ratio with the corresponding 95% confidence interval and p value. Patients who die during follow-up whose deaths are not classified as cardiac deaths will be censored in the above analysis at the time of death.

Since 18F-fluoride uptake is a continuous measure, a potentially more sensitive analysis will explore the relationship between uptake and the risk of suffering an event (cardiac death, recurrent myocardial infarction, or unscheduled coronary revascularisation). This will be performed using the Cox proportional hazards regression model, with (transformed) 18F-fluoride uptake as the only covariate. A range of potential transformations of the 18F-fluoride update will be explored, including fractional polynomials, to find an optimal fit to the data. Once an appropriate transformation for the 18F-fluoride uptake has been identified (as above), the Global Registry of Acute Coronary Events (GRACE) score will be added into the Cox proportional hazards model, to assess the incremental prognostic value of 18F-fluoride over and above conventional clinical risk markers.

11 ADVERSE EVENTS

The Investigator is responsible for the detection and documentation of events meeting the criteria and definitions detailed below.
Full details of contraindications and side effects that have been reported following administration of the IMP are described in the study Investigator's Brochure.

Participants will be instructed to contact their Investigator at any time after consenting to join the trial if any symptoms develop. All adverse events (AE)

as defined in section 11.2 that occur after joining the trial must be reported in detail in the Case Report Form (CRF) or AE form. In the case of an AE, the Investigator should initiate the appropriate treatment according to their medical judgment.

11.1 DEFINITIONS

An **adverse event** (AE) is any untoward medical occurrence in a clinical trial participant which does not necessarily have a causal relationship with an investigational medicinal product (IMP).
An **adverse reaction** (AR) is any untoward and unintended response to an IMP which is related to any dose administered to that participant.
A **serious adverse event** (SAE), **serious adverse reaction** (SAR). Any AE or AR that at any dose:

- results in death of the clinical trial participant
- is life threatening*
- requires in-patient hospitalisation** or prolongation of existing hospitalisation
- results in persistent or significant disability or incapacity
- consists of a congenital anomaly or birth defect
- results in any other significant medical event not meeting the criteria above

*Life-threatening in the definition of an SAE or SAR refers to an event where the participant was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe.

**Any hospitalisation that was planned prior to randomisation will not meet SAE criteria. Any hospitalisation that is planned post randomisation will meet the SAE criteria.

A suspected unexpected serious adverse reaction (SUSAR) is any AR that is classified as serious and is suspected to be caused by the IMP, that it is not consistent with the information about the IMP in the Investigator's Brochure.

11.2 IDENTIFYING AEs AND SAEs

There will be a 48 hour time window to collect and record all AEs and SAEs which occur as a result of the 18F-fluoride intravenous injection and which occur during the 18F-fluoride PET and CT angiogram scanning visits including administration of the beta blockade, glyceryl trinitrate, contrast agent and radiation exposure. Patients attending the scanning visits will be asked to contact the research team if they experience any untoward effects within 48 hours of the scans. Only AEs/SAEs experienced during the scan and selfreported within 48 hours of the PET and /or CT scan will be recorded in the AE log and SAE form, respectively. Any AEs or SAEs out with the above

definitions will not be recorded during the study although data on hospitalisations and deaths will be collected for long-term outcomes.

11.3 RECORDING AEs AND SAEs

Any pre-existing medical conditions (i.e. existed prior to informed consent) are not adverse events but should be recorded as medical history in the CRF. A pre-existing medical condition should only be recorded as an adverse event if the condition worsens during the study. The event term should clearly document that the condition has worsened from baseline.

When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g. hospital notes, laboratory and diagnostic reports) related to the event. The Investigator will then record all relevant information in the CRF and on the SAE form (if the AE meets the criteria of serious).
Information to be collected includes dose, type of event, onset date, Investigator assessment of severity and causality, date of resolution as well as treatment required, investigations needed and outcome.

11.4 ASSESSMENT OF AEs AND SAEs

Seriousness, causality, severity and expectedness will be assessed by the Principal Investigator. Cases that are considered serious, possibly, probably or definitely related to IMP and unexpected (i.e. SUSARs) will be unblinded.
The Investigator is responsible for assessing each AE.
The Chief Investigator may not downgrade an event that has been assessed by an Investigator as an SAE or SUSAR, but can upgrade an AE to an SAE, SAR or SUSAR if appropriate.

11.4.1 Assessment of Seriousness

The Investigator will make an assessment of seriousness as defined in Section 11.1.

11.4.2 Assessment of Causality

The Investigator will make an assessment of whether the AE/SAE is likely to be related to the IMP according to the definitions below.

- Unrelated: where an event is not considered to be related to the IMP.
- Possibly Related: The nature of the event, the underlying medical condition, concomitant medication or temporal relationship make it possible that the AE has a causal relationship to the study drug. The assessment of causality will be made against the reference safety information found in the Investigator's Brochure.

Where non Investigational Medicinal Products (NIMPs) e.g. rescue/escape drugs are given: if the AE is considered to be related to an interaction between the IMP and the NIMP, or where the AE might be linked to either the IMP or the NIMP but cannot be clearly attributed to either one of these, the event will be considered as an AR. Alternative causes such as natural history of the underlying disease, other risk factors and the temporal relationship of the event

to the treatment should be considered and investigated. The blind should not be broken for the purpose of making this assessment.

11.4.3 Assessment of Expectedness

If an event is judged to be an AR, the evaluation of expectedness will be made based on knowledge of the reaction and the relevant product information documented in the IB.
The event may be classed as either:
Expected: the AR is consistent with the toxicity of the IMP listed in the IB.
Unexpected: the AR is not consistent with the toxicity in the IB.

11.4.4 Assessment of Severity

The Investigator will make an assessment of severity for each AE/SAE and record this on the CRF or SAE form according to one of the following categories:
Mild: an event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
Moderate: an event that is sufficiently discomforting to interfere with normal everyday activities.
Severe: an event that prevents normal everyday activities.
Note: the term 'severe', used to describe the intensity, should not be confused with 'serious' which is a regulatory definition based on participant/event outcome or action criteria. For example, a headache may be severe but not serious, while a minor stroke is serious but may not be severe.

11.5 REPORTING OF SAEs/SARs/SUSARs

Once the Investigator becomes aware that an SAE has occurred in a study participant, the information will be reported to the ACCORD Research Governance & QA Office **immediately or within 24 hours**. If the Investigator does not have all information regarding an SAE, they should not wait for this additional information before notifying ACCORD. The SAE report form can be updated when the additional information is received.
The SAE report will provide an assessment of causality and expectedness at the time of the initial report to ACCORD according to Sections 11.4.2, Assessment of Causality and 11.4.3, Assessment of Expectedness. The SAE form will be transmitted by fax to ACCORD on **+44 (0)131 242 9447** or may be transmitted by hand to the office or submitted via email to safety@accord.scot. Only forms in a pdf format will be accepted by ACCORD via email.
Where missing information has not been sent to ACCORD after an initial report, ACCORD will contact the investigator and request the missing information. All reports faxed to ACCORD and any follow up information will be retained by the Investigator in the Investigator Site File (ISF).

11.6 REGULATORY REPORTING REQUIREMENTS

The ACCORD Research Governance & QA Office is responsible for pharmacovigilance reporting on behalf of the co-sponsors (Edinburgh University and NHS Lothian).

The ACCORD Research Governance & QA Office has a legal responsibility to notify the regulatory competent authority and relevant ethics committee (Research Ethics Committee (REC) that approved the trial). Fatal or life threatening SUSARs will be reported no later than 7 calendar days and all other SUSARs will be reported no later than 15 calendar days after ACCORD is first aware of the reaction.
ACCORD will inform Investigators at participating sites of all SUSARs and any other arising safety information.
An Annual Safety Report/Development Safety Update Report will be submitted, by ACCORD, to the regulatory authorities and RECs listing all SARs and SUSARs.

11.7 FOLLOW UP PROCEDURES

After initially recording an AE or recording and reporting an SAE, the Investigator should make every effort to follow each event until a final outcome can be recorded or reported as necessary. Follow up information on an SAE will be reported to the ACCORD office.
If, after follow-up, resolution of an event cannot be established, an explanation should be recorded on the CRF, AE log or SAE form.

12 PREGNANCY

Women who are pregnant, breastfeeding or of child-bearing potential (women who have experienced menarche, are pre-menopausal and have not been sterilised) will not be enrolled into the trial.

13 TRIAL MANAGEMENT AND OVERSIGHT ARRANGEMENTS

13.1 TRIAL MANAGEMENT GROUP

The trial will be coordinated by a Project Management Group, consisting of the grant holders (Chief Investigator and Principal Investigator in Edinburgh) and site leads.
The Principal Investigator will oversee the study and will be accountable to the Chief Investigator. The Principal Investigator will be responsible for checking the CRFs for completeness, plausibility and consistency. Any queries will be resolved by the Investigator or delegated member of the trial team.
A Delegation Log will be prepared for each site, detailing the responsibilities of each member of staff working on the trial.

13.2 TRIAL STEERING COMMITTEE

A Trial Steering Committee (TSC) will be established to oversee the conduct and progress of the trial. Contact details of the TSC are detailed in a separate TSC charter. The review of SAEs will be added to the TSC agenda to ensure that appropriate action is taken if any safety issues arise.

13.3 DATA MONITORING COMMITTEE

This is an observational open label clinical cohort study involving an established radiotracer with over 40 years of experience. The result of the 18F-fluoride positron emission tomography and computed tomography coronary angiogram will not be fed back to the clinical team responsible for the patients care (except for clinically important incidental findings). For this reason, an independent Data Monitoring Committee (DMC) will not be convened for this study and all study serious adverse events will be reported to the sponsor and will be discussed by the TSC.

13.4 INSPECTION OF RECORDS

Investigators and institutions involved in the study will permit trial related monitoring and audits on behalf of the sponsor, REC review, and regulatory inspection(s). In the event of an audit or monitoring, the Investigator agrees to allow the representatives of the sponsor direct access to all study records and source documentation. In the event of regulatory inspection, the Investigator agrees to allow inspectors direct access to all study records and source documentation.

13.5 RISK ASSESSMENT

A study specific risk assessment has been performed by representatives of the co-sponsors, ACCORD monitors and the QA group, in accordance with ACCORD governance and sponsorship SOPs. Input has been sought from the Chief Investigator or designee. The outcomes of the risk assessment were the basis from which monitoring plans and audit plans were created. The risk assessment outcomes also indicated which risk adaptions (delete if no adaptations were possible) could be incorporated into to trial design.

13.6 BENEFIT/RISK BALANCE

13.6.1 Benefits

Patients may also benefit from additional procedures and investigations that they will undergo as part of the study. This will include closer medical supervision and non-invasive imaging investigations that may identify important incidental findings.

13.6.2 Risks

There are some potential hazards of the non-invasive investigations that we will perform as part of the trial. The main issues relate to exposure to ionising radiation and contrast agent administration. We have a well-developed protocol for cardiac positron emission and computed tomography imaging that minimises radiation exposure and has clear procedures for managing adverse contrast reactions. We anticipate that the total research protocol dose (TPRD) radiation exposure not exceed 34 mSv. The TRPD includes radiation exposure from both the positron emission tomography and computed tomography scans combined. The estimated associated risk of developing fatal cancer is proportional to dose. Using a risk of 5% per Sv [ARSAC Notes] in a healthy

population in this age group the estimated associated risk of developing fatal cancer as a result of this exposure is in the region 1 in 650. This risk can be classified as moderate. It is likely that in a population of any patients in the age group 50+ the cancer risk is lower than 5% per Sv. For comparison the average annual background radiation dose arising from natural sources of ionising radiation in the environment in the UK is 2.2 mSv. The TRPD of 34 mSv incurred in this study is approximately 15 times annual background radiation from natural sources. This can be compared with other commonly used cardiovascular imaging techniques, such as nucleotide myocardial perfusion imaging (15-20 mSv) and diagnostic coronary angiography (7 mSv) [Einstein *et al*, 2007].
The risks of exposure to the contrast medium include allergic reactions and impairment of kidney function. Amongst patients with moderate-to-severe chronic kidney disease, there is a 2-4% risk of kidney impairment after computed tomography angiography [Barrett *et al*, 2006]. The risk of contrast exposure in this study will be minimised by exclusion of high-risk patients who have significant kidney disease (estimated glomerular filtration rate <30 mL/min/1.73m^2).

13.7 STUDY MONITORING AND AUDIT

ACCORD clinical trial monitors, or designees, will perform monitoring activities in accordance with the study monitoring plan. This will involve on-site visits and central monitoring activities as necessary (delete where not required). ACCORD QA personnel, or designees, will perform study audits in accordance with the study audit plan. This will involve investigator site audits, study management audits and facility (including 3rd parties) audits as necessary (delete where not required).

14 GOOD CLINICAL PRACTICE

14.1 ETHICAL CONDUCT

The study will be conducted in accordance with the principles of the International Conference on Harmonisation Tripartite Guideline for Good Clinical Practice (ICH GCP).
A favorable ethical opinion will be obtained from the appropriate REC and local R&D approval will be obtained prior to commencement of the study.

14.2 REGULATORY COMPLIANCE

The study will not commence until a Clinical Trial Authorisation (CTA) is obtained from the appropriate Regulatory Authority. The protocol and study conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004, as amended.

14.3 INVESTIGATOR RESPONSIBILITIES

The Investigator is responsible for the overall conduct of the study at the site and compliance with the protocol and any protocol amendments. In accordance

with the principles of ICH GCP, the following areas listed in this section are also the responsibility of the Investigator. Responsibilities may be delegated to an appropriate member of study site staff.

14.3.1 Informed Consent

The Investigator is responsible for ensuring informed consent is obtained before any protocol specific procedures are carried out. The decision of a participant to participate in clinical research is voluntary and should be based on a clear understanding of what is involved.
Participants must receive adequate oral and written information – appropriate Participant Information and Informed Consent Forms will be provided. The oral explanation to the participant will be performed by the Investigator or qualified delegated person and must cover all the elements specified in the Participant Information Sheet and Consent Form.
The participant must be given every opportunity to clarify any points they do not understand and, if necessary, ask for more information. The participant must be given sufficient time to consider the information provided. It should be emphasised that the participant may withdraw their consent to participate at any time without loss of benefits to which they otherwise would be entitled. The participant will be informed and agree to their medical records being inspected by regulatory authorities and representatives of the sponsor(s) but understand that their name will not be disclosed outside the hospital.
The Investigator or delegated member of the trial team and the participant will sign and date the Informed Consent Form(s) to confirm that consent has been obtained. The participant will receive a copy of this document and a copy filed in the Investigator Site File (ISF) and participant's medical notes.

14.3.2 Study Site Staff

The Investigator must be familiar with the IMP, protocol and the study requirements. It is the Investigator's responsibility to ensure that all staff assisting with the study are adequately informed about the IMP, protocol and their trial related duties.

14.3.3 Data Recording

The Principal Investigator is responsible for the quality of the data recorded in the CRF at each Investigator Site. The source data plan identifies which source data correspond to CRF data and states which data are recorded directly into the CRF.

14.3.4 Investigator Documentation

Prior to beginning the study, each Investigator will be asked to provide particular essential documents to the ACCORD Research Governance & QA Office, including but not limited to:

- An original signed Investigator's Declaration (as part of the Clinical Trial Agreement documents);
- Curriculum vitae (CV) signed and dated by the Investigator indicating that it is accurate and current.

The ACCORD Research Governance & QA Office will ensure all other documents required by ICH GCP are retained in a Trial Master File (TMF), where required, and that appropriate documentation is available in local ISFs.

14.3.5 GCP Training

All study staff will hold evidence of GCP training where appropriate.

14.3.6 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records must be identified in a manner designed to maintain participant confidentiality. All records must be kept in a secure storage area with limited access. Identifiable clinical information will not be released without the written permission of the participant. The Investigator and study site staff involved with this study may not disclose or use for any purpose other than performance of the study, any identifiable data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

14.3.7 Data Protection

All Investigators and study site staff involved with this study must comply with the requirements of the appropriate data protection legislation (including where applicable the General Data Protection Regulation with regard to the collection, storage, processing and disclosure of personal information. Access to personal information will be restricted to individuals from the research team treating the participants, representatives of the sponsor(s) and representatives of regulatory authorities.
Computers used to collate the data will have limited access measures via user names and passwords.
Published results will not contain any personal data that could allow identification of individual participants.

15 STUDY CONDUCT RESPONSIBILITIES

15.1 PROTOCOL AMENDMENTS

Any changes in research activity, except those necessary to remove an apparent, immediate hazard to the participant in the case of an urgent safety measure, must be reviewed and approved by the Chief Investigator. Amendments to the protocol must be submitted in writing to the appropriate REC, Regulatory Authority and local R&D for approval prior to participants being enrolled into an amended protocol.

15.2 PROTOCOL VIOLATIONS AND DEVIATIONS

Prospective protocol deviations, i.e. protocol waivers, will not be approved by the sponsors and therefore will not be implemented, except where necessary to eliminate an immediate hazard to study participants. If this necessitates a

subsequent protocol amendment, this should be submitted to the REC, Regulatory Authority and local R&D for review and approval if appropriate. Protocol deviations will be recorded in a protocol deviation log and logs will be submitted to the sponsors every 3 months. Each protocol violation will be reported to the sponsor within 3 days of becoming aware of the violation. Deviation logs / violation forms will be transmitted via email to QA@accord.scot Only forms in a pdf format will be accepted by ACCORD via email. Forms may also be sent by fax to ACCORD on +44 (0)131 242 9447 or may be submitted by hand to the office. Where missing information has not been sent to ACCORD after an initial report, ACCORD will contact the Investigator and request the missing information. The Investigator must respond to these requests in a timely manner

15.3 SERIOUS BREACH REQUIREMENTS

A serious breach is a breach which is likely to effect to a significant degree: (a) the safety or physical or mental integrity of the participants of the trial; or (b) the scientific value of the trial.

If a potential serious breach is identified by the Chief investigator, Principal Investigator or delegates, the co-sponsors (QA@accord.scot) must be notified within 24 hours. It is the responsibility of the co-sponsors to assess the impact of the breach on the scientific value of the trial, to determine whether the incident constitutes a serious breach and report to regulatory authorities and research ethics committees as necessary.

15.4 STUDY RECORD RETENTION

All study documentation will be kept for a minimum of 5 years from the protocol defined end of study point. When the minimum retention period has elapsed, study documentation will not be destroyed without permission from the sponsor.

15.5 END OF STUDY

It is anticipated that the study will last 6 years: four years for recruitment and two years of follow-up. The end of study is defined as the last participant's last visit.

The Investigators and/or the trial steering committee and/or the co-sponsor(s) have the right at any time to terminate the study for clinical or administrative reasons.

The end of the study will be reported to the REC, Regulatory Authority, R&D Office(s) and co-sponsors within 90 days, or 15 days if the study is terminated prematurely. The Investigators will inform participants of the premature study closure and ensure that the appropriate follow up is arranged for all participants involved. End of study notification will be reported to the co-sponsors via email to resgov@ed.ac.uk.

In accordance with ACCORD SOP CR011, a Clinical Study Report (CSR) will be provided to the Sponsor (QA@accord.scot) and REC within 1 year of the end of the study.

Upon completion of the study, the Investigator will upload clinical trial results onto the EudraCT database on behalf of the Sponsor.

15.6 INSURANCE AND INDEMNITY

The co-sponsors are responsible for ensuring proper provision has been made for insurance or indemnity to cover their liability and the liability of the Chief Investigator and staff.

The following arrangements are in place to fulfil the co-sponsors' responsibilities:

- The Protocol has been designed by the Chief Investigator and researchers employed by the University and collaborators. The University has insurance in place (which includes no-fault compensation) for negligent harm caused by poor protocol design by the Chief Investigator and researchers employed by the University.
- Sites participating in the study will be liable for clinical negligence and other negligent harm to individuals taking part in the study and covered by the duty of care owed to them by the sites concerned. The cosponsors require individual sites participating in the study to arrange for their own insurance or indemnity in respect of these liabilities.
- Sites which are part of the United Kingdom's Nation Health Service will have the benefit of NHS Indemnity.
- Sites out with the United Kingdom will be responsible for arranging their own indemnity or insurance for their participation in the study, as well as for compliance with local law applicable to their participation in the study.
- The manufacturer supplying IMP has accepted limited liability related to the manufacturing and original packaging of the study drug and to the losses, damages, claims or liabilities incurred by study participants based on known or unknown Adverse Events which arise out of the manufacturing and original packaging of the study drug, but not where there is any modification to the study drug (including without limitation re-packaging and blinding).

16 REPORTING, PUBLICATIONS AND NOTIFICATION OF RESULTS

16.1 AUTHORSHIP POLICY

Ownership of the data arising from this study resides with the study team. On completion of the study, the study data will be analysed and tabulated, and a clinical study report will be prepared in accordance with appropriate regulatory guidelines.

16.2 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 clinics (where appropriate and according to their discretion).

16.3 PEER REVIEW

The study has undergone national and international peer review by the Wellcome Trust. There is additional peer review from the Trial Steering Committee and the Edinburgh Clinical Trials Unit as well as the study sponsors.

17 REFERENCES

ARSAC Notes for Guidance on the Clinical Administration of Radiopharmaceuticals and Use of Sealed Radioactive Sources -March 2006 (updated 2014) Appendix VII

Barrett BJ, Katzberg RW, Thomsen HS, Chen N, Sahani D, Soulez G, Heiken JP, Lepanto L, Ni ZH, Ni ZH, Nelson R. Contrast-induced nephropathy in patients with chronic kidney disease undergoing computed tomography: a double-blind comparison of iodixanol and iopamidol. *Invest Radiol*. 2006;41:815-821.

Davies M. The pathophysiology of acute coronary syndromes. Heart. 2000;83:361-6.

Detrano R, Guerci AD, Carr JJ, et al. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. *N Engl J Med* 2008;358:13361345.

Dweck MR, Chow MW, Joshi NV, Williams MC, Jones C, Fletcher AM, Richardson H, White A, McKillop G, van Beek EJ, Boon NA, Rudd JH, Newby DE. Coronary arterial 18f-sodium fluoride uptake: A novel marker of plaque biology. *J Am Coll Cardiol*. 2012b;59:1539-1548

Dweck MR, Jones C, Joshi NV, Fletcher AM, Richardson H, White A, Marsden M, Pessotto R, Clark JC, Wallace WA, Salter DM, McKillop G, van Beek EJ, Boon NA, Rudd JH, Newby DE. Assessment of valvular calcification and inflammation by positron emission tomography in patients with aortic stenosis. *Circulation*. 2012a;125:76-86

Dweck MR, Khaw HJ, Sng GK, Luo EL, Baird A, Williams MC, Makiello P, Mirsadraee S, Joshi NV, van Beek EJ, Boon NA, Rudd JH, Newby DE. Aortic stenosis, atherosclerosis, and skeletal bone: Is there a common link with calcification and inflammation? *Eur Heart J*. 2013;34:1567-1574.

Einstein AJ, Moser KW, Thompson RC, Cerqueira MD, Henzlova MJ. Radiation dose to patients from cardiac diagnostic imaging. *Circulation*. 2007;116:12901305.

Joshi NV, Vesey AT, Williams MC, Shah ASV, Calvert PA, Craighead FHM, Yeoh SE, Wallace W, Salter D, Fletcher AM, van Beek EJR, Flapan AD, Uren NG, Behan MWH, Cruden NLM, Mills NL, Fox KAA, Rudd JHF, Marc R Dweck MR, Newby DE. 18F-Fluoride positron emission tomography identifies ruptured and high-risk coronary atherosclerotic plaques. *Lancet* 2014;383:705-713.

Kwiecinski J, Cadet S, Daghem M, Lassen ML, Dey D, Dweck MR, Berman DS, Newby DE, Slomka PJ. Whole-vessel coronary (18)F-sodium fluoride PET for assessment of the global coronary microcalcification burden. Eur J Nucl Med Mol Imaging. 2020a;47:1736-1745.

Kwiecinski J, Tzolos E, Adamson PD, Cadet S, Moss AJ, Joshi N, Williams MC, van Beek EJR, Dey D, Berman DS, Newby DE, Slomka PJ, Dweck MR. Coronary (18)F-Sodium Fluoride Uptake Predicts Outcomes in Patients With Coronary Artery Disease. J Am Coll Cardiol. 2020b;75:3061-3074. Mann J, Davies MJ. Mechanisms of progression in native coronary artery disease: role of healed plaque disruption. *Heart*. 1999;82:265-8.

Mills NL, Churchhouse AM, Lee KK, et al. Implementation of a sensitive troponin I assay and risk of recurrent myocardial infarction and death in patients with suspected acute coronary syndrome. *JAMA* 2011;305:1210-16.

Mills NL, Lee KK, McAllister DA, Churchhouse AMD, MacLeod M, Stoddard M, Walker S, Denvir MA, Fox KAA, Newby DE. Implications of lowering the threshold of plasma troponin in the diagnosis of myocardial infarction: a cohort study. *BMJ* 2012;344:e1533.

Omland T, Pfeffer MA, Solomon SD, de Lemos JA, Rosjo H, Saltyte Benth J, Maggioni A, Domanski MJ, Rouleau JL, Sabatine MS, Braunwald E. Prognostic value of cardiac troponin i measured with a highly sensitive assay in patients with stable coronary artery disease. *J Am Coll Cardiol*. 2013;61:1240-1249

Rioufol G, Gilard M, Finet G, Ginon I, Boschat J, André-Fouët X. Evolution of spontaneous atherosclerotic plaque rupture with medical therapy: long-term follow-up with intravascular ultrasound. *Circulation*. 2004;110:2875-80. Shah ASV, Griffiths M, Lee KK, McAllister, DA, Hunter AL, Cruikshank A, Alan Reid A, Stoddart M, Strachan F, Walker S, Collinson PO,Apple FS, Gray AJ, Fox KAA, Newby DE, Mills NL. High-sensitivity cardiac troponin and the under diagnosis of myocardial infarction in women. *ESC Hotline 2013.*

Thomas GS, Haraszti RA. A new frontier in atherosclerotic coronary imaging. Lancet. 2014;383:674-5.

Thompson RC, Allam AH, Lombardi GP, Wann LS, Sutherland ML, Sutherland JD, Soliman MA, Frohlich B, Mininberg DT, Monge JM, Vallodolid CM, Cox SL, Abd el-Maksoud G, Badr I, Miyamoto MI, el-Halim Nur el-Din A, Narula J, Finch CE, Thomas GS. Atherosclerosis across 4000 years of human history: the Horus study of four ancient populations. Lancet. 2013;381:1211-22.

Tzolos E, Kwiecinski J, Lassen ML, Cadet S, Adamson PD, Moss AJ, Joshi N, Williams MC, van Beek EJR, Dey D, Berman DS, Dweck MR, Newby DE, Slomka PJ. Observer repeatability and interscan reproducibility of 18F-sodium fluoride coronary microcalcification activity. J Nucl Cardiol. 2020; in press. doi: 10.1007/s12350-020-02221-1.

FIGURE 1. Focal 18F-Fluoride Uptake in Patients with Myocardial Infarction and Stable Angina

Patient with acute ST-segment elevation myocardial infarction with (A) proximal occlusion (red arrow) of the left anterior descending artery on invasive coronary angiography and (B) intense focal 18F-fluoride uptake (yellow-red) at the site of the culprit plaque (red arrow) on the combined positron emission and computed tomogram.

Patient with anterior non-ST-segment elevation myocardial infarction with (C) culprit (red arrow; left anterior descending artery) and bystander non-culprit (white arrow; circumflex artery) lesions on invasive coronary angiography that were both stented during the index admission. Only the culprit lesion had increased 18F-fluoride uptake on combined positron emission and computed tomography (D) following percutaneous coronary intervention.

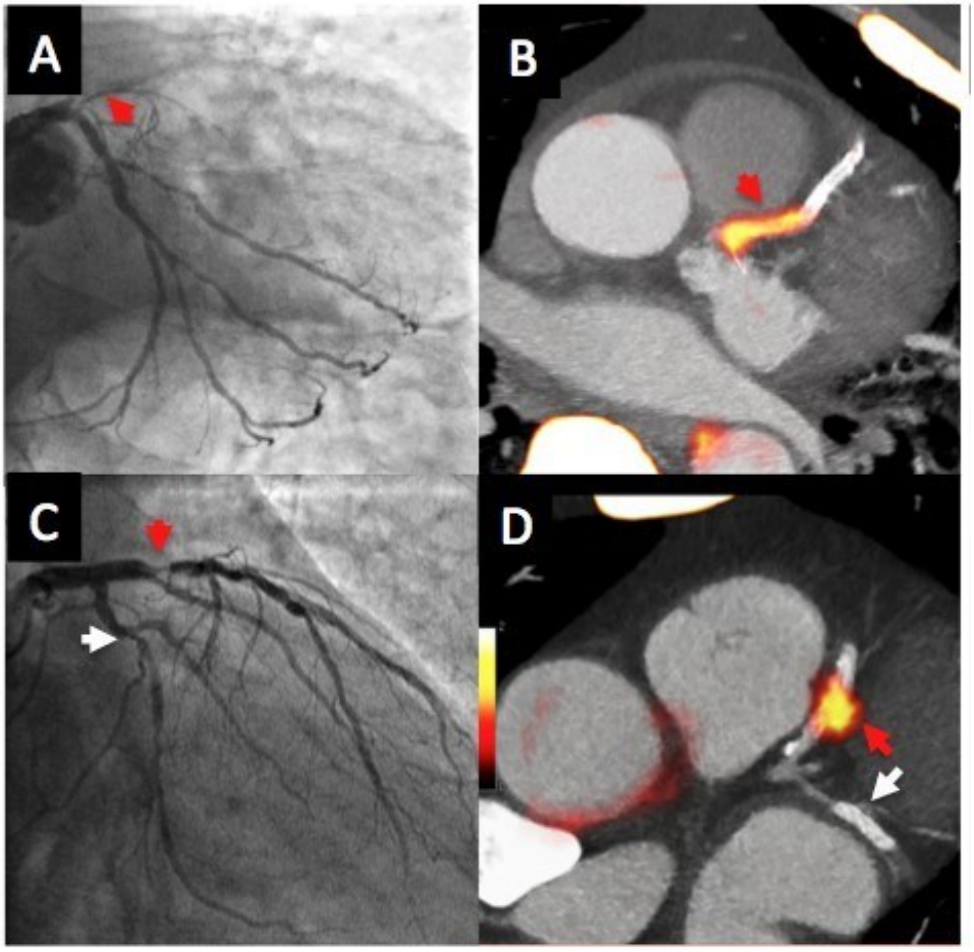


FIGURE 2. Patients with Stable Angina and 18F-Fluoride Uptake

Representative examples for 18F-fluoride uptake in patients with stable angina. Panels A-D, computed tomography coronary angiograms; panels E-H, 18Ffluoride positron emission tomograms; and panels I-L, fused positron emission tomograms and computed tomography coronary angiograms.

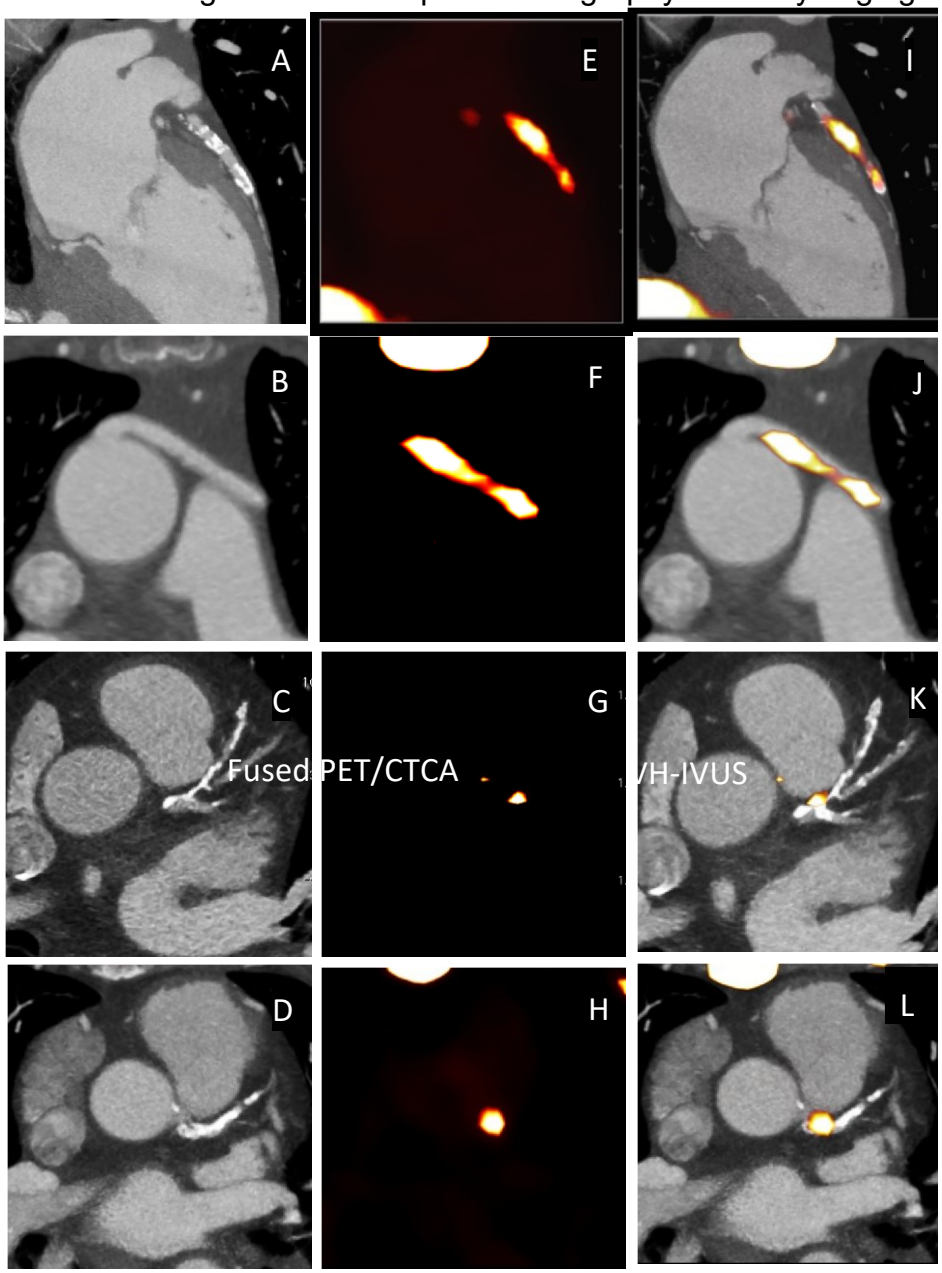
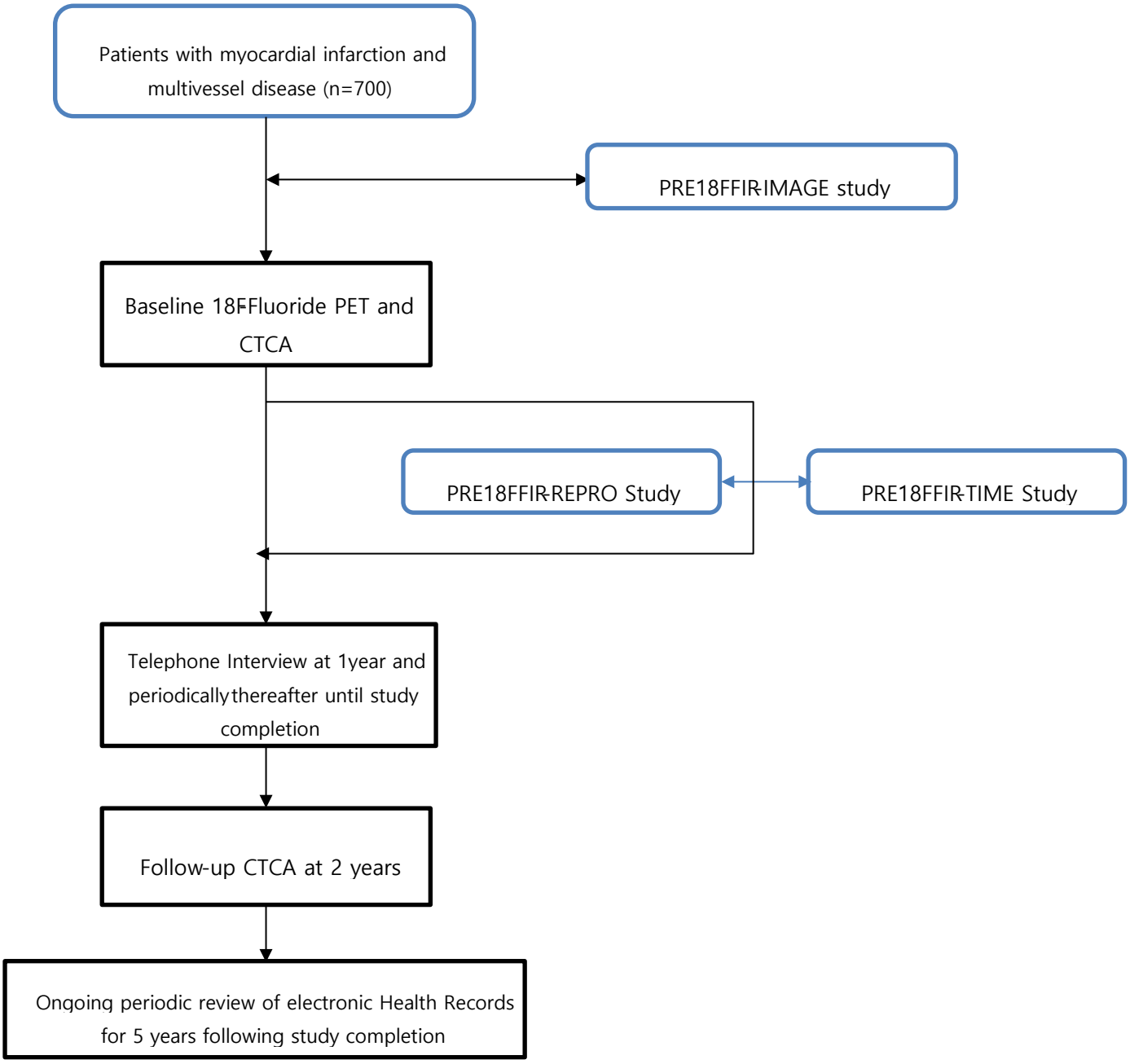


FIGURE 3. Patient Flow Chart



PRE^{18}FFIR

Prediction of Recurrent Events with ^{18}F-Fluoride to Identify Rupture and High-risk Coronary Artery Plaques in Patients with Myocardial Infarction

Statistical Analysis Plan

Version No	1.0
Date Finalised	23 March 2022
Author(s)	Lumine Na (to Feb 2016), Steff Lewis (after Feb 2016)
Chief Investigator	Dave Newby
Chief Investigator Email Address	d.e.newby@ed.ac.uk

Signatures	
Trial Statistician:	**Date:**
Chief Investigator:	**Date:**

Document Control

Version No	Date	Summary of Revisions
1.0	23 March 2022	Finalised SAP created
Draft versions prior to official numbering of V1.0	09 Feb 2016	Draft SAP created, and edited following comments from chief investigator and trial statistician

Table of Contents

List of Abbreviations

Abbreviation	Full name
^{18}F-fluoride	^{18}F-sodium fluoride
ACE	Angiotensin Converting Enzyme
ARB	Angiotensin Receptor Blocker
CABG	Coronary Artery Bypass Graft
CI	Confidence Interval
CMA	Coronary Microcalcification Activity
CMA status	Binary classification of whether or not a participant has coronary microcalcification activity
CT	Computed Tomography
CONSORT	Consolidated Standards of Reporting Trials
ECTU	Edinburgh Clinical Trials Unit
GRACE	Global Registry of Acute Coronary Events
IMP	Investigational Medicinal Product
LMS	Left Main Stem coronary artery
MBq	Megabecquerel
MedDRA	Medical Dictionary for Regulatory Activities
MI	Myocardial Infarction
mSv	Millisievert
N	Number of participants with an observation
NIMP	Non-Investigational Medicinal Product
NSTEMI	Non-ST Elevation Myocardial Infarction
PCI	Percutaneous Coronary Intervention
PET	Positron Emission Tomography
PRE^{18}FFIR	Trial acronym for 'Prediction of Recurrent Events with ^{18}F-Fluoride to Identify Ruptured and High-risk Coronary Artery Plaques with Myocardial Infarction'
Q1	Lower quartile
Q3	Upper quartile
SAP	Statistical Analysis Plan
SD	Standard Deviation
STEMI	ST Elevation Myocardial Infarction
TIA	Transient Ischaemic Attack

1. Introduction

This document details the criteria to be used for the definition of the analysis populations and the statistical methodology for analysis for the PRE^{18}FFIR trial. This SAP covers the main PRE^{18}FFIR protocol. It does not relate to the PRE^{18}FFIR substudies that are covered by separate protocols – these will not be analysed by the ECTU statistics team. In addition, this SAP does not cover the exploratory objectives in the protocol – these will not be analysed by the ECTU statistics team.

PRE^{18}FFIR is a prospective multi-centre observational cohort study to investigate the associations between coronary ^{18}F-fluoride uptake and disease progression and clinical outcomes, in patients with recent myocardial infarction and multi-vessel coronary artery disease. All participants receive the IMP, ^{18}F-sodium fluoride (^{18}F-fluoride), and undergo ^{18}F-fluoride positron emission tomography to determine their level of ^{18}F-fluoride uptake. The aim is to recruit 700 participants.

This document has been compiled according to the Edinburgh Clinical Trials Unit (ECTU) standard operating procedure "Statistical Analysis Plans" ECTU_ST_04 and has been written based on information contained in the PRE^{18}FFIR study protocol version 11.0, dated 24 February 2022.

2. Statistical Methods section from the protocol

The primary analysis will be performed by calculating Kaplan-Meier estimates of the 'survival' curves for the time to first event (cardiac death, recurrent myocardial infarction, or unscheduled coronary revascularisation) during follow up, and comparing these estimates for participants with and without coronary microcalcification activity using a log-rank test. The results will be expressed as the estimated hazard ratio with the corresponding 95% confidence interval and p value. Participants who die during follow-up whose deaths are not classified as cardiac deaths will be censored in the above analysis at the time of death.

Since 18F-fluoride uptake is a continuous measure, a potentially more sensitive analysis will explore the relationship between uptake and the risk of suffering an event (cardiac death, recurrent myocardial infarction, or unscheduled coronary revascularisation). This will be performed using the Cox proportional hazards regression model, with (transformed) 18F-fluoride uptake as the only covariate. A range of potential transformations of the 18F-fluoride uptake will be explored, including fractional polynomials, to find an optimal fit to the data.

Once an appropriate transformation for the 18F-fluoride uptake has been identified (as above), the Global Registry of Acute Coronary Events (GRACE) score will be added into the Cox proportional hazards model, to assess the incremental prognostic value of 18F-fluoride over and above conventional clinical risk markers.

There are 3 secondary endpoints in the protocol that will be analysed later (not by ECTU), and will not form part of this SAP:

- ^{18}F-fluoride uptake localisation to culprit plaque causing the index myocardial infarction
- Territory of subsequent myocardial reinfarction
- Coronary artery plaque progression by computed tomography coronary angiography. Plaque volume, composition and calcification

3. Overall Statistical Principles

In general terms, categorical data will be presented using counts (N) and percentages (%), whilst continuous variables will be presented using the mean, median, standard deviation (SD), minimum, maximum, lower and upper quartiles [Q1, Q3], and number of participants with an observation (N). Data will be split by time point where applicable.

In general, analyses will be unadjusted, unless otherwise specified.

All applicable statistical tests will be 2-sided and will be performed using a 5% significance level. 95% (2-sided) confidence intervals (CIs) will be presented. All analyses are seeking to show a difference, rather than equivalence or non-inferiority.

All analyses and data manipulations will be carried out using the most up to date version of SAS available [1].

There are no pre-planned subgroup analyses.

Note: Throughout this document, "study entry" is defined as the date a participant had the baseline PET-CT scan as captured on the baseline visit case report form. "CMA status" is used to denote the binary classification of whether or not a participant had coronary microcalcification activity. Those participants with CMA>0 are defined as having coronary microcalcification activity, and those with CMA=0 as not having any coronary microcalcification activity.

Note: Due to Covid-19, some follow ups at two years will have the imaging visit and the rest of the follow up done at different times. The database only captures one date. This should be for the nonimaging follow-up. It should not be assumed that two year follow up dates relate to the imaging visits.

4. Analysis Populations

Scanned population

This population will include all patients who had the baseline PET-CT scan completed.

Analysis population

This population will include all patients who had the baseline PET-CT scan and CMA status available from analysis of scan images.

All analyses will be performed on the analysis population unless otherwise specified.

Safety population

This population will include all patients who received the radioactive tracer.

5. List of Analyses

5.1 Recruitment and retention

The data to construct a CONSORT flow chart will be provided, as far as they are available in the study database. We will aim to tabulate: number of patients who were approached to participate in the study; number of patients who were excluded (with a tabulation of reasons for exclusion); number of patients who had the screening visit; number of patients who were excluded (with a tabulation of reasons for exclusion); number of patients who attended the baseline visit and received the

radioactive tracer [^{18}F-fluoride]; number of patients who received the radioactive tracer and the baseline scan was not started, or started but aborted (with tabulation of reasons); number of patients who had baseline scan completed ; number of participants who withdrew consent including to use of existing data; number of participants with follow-up completed or who died, or had follow-up discontinued prematurely (with tabulation of reasons for premature discontinuation); ; total personyears of follow-up during the study, before Covid-19, and during Covid-19; number of participants included in analysis of primary outcome; number of participants excluded from analysis of primary outcome (with tabulation of reasons). Median, lower and upper quartile, minimum and maximum for duration of follow-up (overall and split by CMA status).

The statistical report will provide for the scanned population, not split by CMA status, a graph of cumulative number of patients recruited into the study over time and the dates the first and last patients were recruited; the number of study sites that recruited patients; a tabulation of the number of patients recruited by each study site.

5.2 Baseline characteristics

The following baseline characteristics will be tabulated by CMA status, and overall. No formal statistical testing will be performed.

Demographics

a. Sex (Male/Female)
b. Age (years) and (18-64, 65-84, ≥85)
c. Body mass index (kg/m^2)

Cardiovascular Risk Factors

d. Smoking status (Never/Ex-smoker/Current)
e. Hypertension or receiving treatment for hypertension (Yes/No)
f. High cholesterol (>5 mmol/L) or on cholesterol lowering treatment prior to admission (Yes/No)
g. Diabetes mellitus (Yes/No) if Yes then type (Type 1/Type 2)

Cardiovascular History

h. Previous diagnosis of ischaemic heart disease prior to admission (Yes/No)
i. Documented history of myocardial infarction prior to admission (Yes/No)
j. Previous PCI prior to admission (Yes/No)
k. Previous CABG (Yes/No)
l. History of peripheral vascular disease in the medical notes (Yes/No)
m. History of stroke or TIA in the medical notes (Yes/No)

Index Admission for Myocardial Infarction

n. Index myocardial infarction (STEMI/NSTEMI)
o. Coronary artery disease (Single vessel without LMS involvement, Single vessel with LMS involvement, Two vessels without LMS involvement, Two vessels with LMS involvement, Three vessels without LMS involvement, Three vessels with LMS involvement)
p. Percutaneous coronary intervention (Yes/No)
q. GRACE score (continuous)

Medication at Time of Screening Visit

N (%) of participants prescribed each of the following categories of medication:

- Aspirin ○ $P2Y_{12}$ antagonist ○ Statin
- Non-statin lipid lowering therapy
- Beta blocker ○ ACE inhibitor or ARB ○ Calcium channel blocker ○ Nitrate ○ Other anti-anginal
- Mineralocorticoid receptor antagonist ○ Other diuretic ○ Anticoagulant
- Insulin
- Oral diabetic medication

5.3 Primary endpoint

5.3.1 Endpoint Definition

Cardiac death or non-fatal recurrent myocardial infarction or unscheduled coronary revascularisation between study entry and up to the end of follow-up. Unscheduled (late) coronary revascularisation is defined as revascularisation >6 weeks after screening visit date.

5.3.2 Primary Analysis

Table: N (%) of participants with primary outcome event by CMA status and overall.

Table: Numbers of participants whose first event was: a cardiac death, a non-fatal recurrent MI, or an unscheduled coronary revascularisation by CMA status and overall.

Figure: Kaplan-Meier estimates of cumulative incidence from analysis of time from study entry to first event of cardiac death or non-fatal recurrent MI or unscheduled coronary revascularisation by CMA status with p-value using a log-rank test. This will be the primary statistical analysis. Participants who do not have a primary outcome event will be censored at the time of non-cardiac death or last follow-up.

Table: Hazard ratio of primary outcome by CMA status, with 95% CI, and associated p-value using Cox regression.

5.3.3 Exploratory analyses of primary outcome

Relationship between CMA, as a continuous measure of ^{18}F-fluoride uptake, and the risk of suffering a primary outcome event will be explored using a Cox proportional hazards regression model with (transformed) CMA as the only explanatory variable. A range of potential transformations of CMA will be explored, including fractional polynomials, to find an optimal fit to the data.

A second Cox regression model with (transformed) GRACE score as the only explanatory variable will be used to explore the relationship between this risk score and the primary outcome. A range of potential transformations of GRACE score will be explored to find an optimal fit to the data. The precise version of the GRACE score used, and any necessary details of calculation, will be specified in the statistical report.

A third Cox regression model with both GRACE score and CMA as explanatory variables, transformed where appropriate, will be used to assess the incremental prognostic value of CMA over and above a conventional clinical risk marker.

Table: Hazard ratio of primary outcome for each explanatory variable, with 95% CI, and associated pvalue from each Cox regression model. Also for each model the C-statistic, with 95% CI, as a measure of the discriminatory ability.

5.4 Secondary endpoints

5.4.1 All-cause death

Table: N (%) of participants with all cause death by CMA status and overall.

Figure: Kaplan-Meier estimates of cumulative incidence from analysis of time from study entry to death by CMA status with p-value using a log-rank test. Participants who do not have a relevant outcome event will be censored at the time of last follow-up.

Table: Hazard ratio of all cause death by CMA status, with 95% CI, and associated p-value using Cox regression.

5.4.2 Cardiac death

Table: N (%) of participants with cardiac death by CMA status and overall.

Figure: Kaplan-Meier estimates of cumulative incidence from analysis of time from study entry to cardiac death by CMA status with p-value using a log-rank test. Participants who do not have a relevant outcome event will be censored at the time of non-cardiac death or last follow-up.

Table: Hazard ratio of cardiac death by CMA status, with 95% CI, and associated p-value using Cox regression.

5.4.3 Non-fatal recurrent myocardial infarction

Table: N (%) of participants with non-fatal recurrent myocardial infarction by CMA status and overall.

Figure: Kaplan-Meier estimates of cumulative incidence from analysis of time from study entry to first non-fatal recurrent MI by CMA status with p-value using a log-rank test. Participants who do not have a relevant outcome event will be censored at the time of death or last follow-up.

Table: Hazard ratio of non-fatal recurrent myocardial infarction by CMA status, with 95% CI, and associated p-value using Cox regression.

5.4.4 Unscheduled coronary revascularisation

Table: N (%) of participants with unscheduled coronary revascularisation by CMA status and overall.

Figure: Kaplan-Meier estimates of cumulative incidence from analysis of time from study entry to first unscheduled coronary revascularisation by CMA status with p-value using a log-rank test. Participants who do not have a relevant outcome event will be censored at the time of death or last follow-up.

Table: Hazard ratio of unscheduled coronary revascularisation by CMA status, with 95% CI, and associated p-value using Cox regression.

5.5 Safety analyses

5.5.1 To determine the radiation exposure and effective dose associated with ^{18}F-fluoride positron emission tomography and computed tomography coronary angiography

The following will be presented for the scanned population. Details of radiation exposure for patients in the safety population who are not in the scanned population will be put in a footnote.

Table: mean, standard deviation, median, lower and upper quartile, minimum, and maximum of injected ^{18}F-fluoride activity (MBq) and effective radiation dose (mSv) from administration of radioactive tracer at baseline visit. The effective radiation dose (mSv) will be calculated from the administered dose using the conversion factor of 0.024 mSv/MBq. Mean, standard deviation, median, lower and upper quartile, minimum, and maximum of dose-length product (Gy□cm) and effective radiation dose (mSv) from computed tomography component of the scans at baseline visit. The effective radiation dose (mSv) will be calculated from the dose-length product using the conversion factor of 0.014 mSv/Gy□cm. Mean standard deviation, median, lower and upper quartile, minimum, and maximum of total effective radiation dose (mSv) at baseline visit.

The following will be presented for the participants who had a CT scan at 2 years.

Table: mean, standard deviation, median, lower and upper quartile, minimum, and maximum of doselength product (mGy□cm) and effective radiation dose (mSv) from the scans at 2-year visit. The effective radiation dose (mSv) will be calculated from the dose-length product using the conversion factor of 0.014 mSv/Gy□cm.

These tabulations will not be split by CMA status.

5.5.2. To identify adverse events associated with ^{18}F-fluoride positron emission tomography and computed tomography angiography

The following will be presented for the safety population.

Table: summary of adverse events within 48 hours of baseline visit showing both numbers and percentages of patients with events, and number of events. Also present these numbers split by whether events are serious or non-serious, and within seriousness by (i) causality (unrelated or possibly related for IMP and NIMP) and MedDRA preferred term (or an alternative classification), and (ii) MedDRA system organ class and MedDRA preferred term (or an alternative classification). No formal statistical analysis will be performed.

Listings with details of each serious adverse event and each non-serious adverse event, separately for those within 48 hours of baseline visit and 48 hours of 2-year visit.

These tabulations and listings will not be split by CMA status.

5.6. Deviations, Violations

Listing of protocol violations and listing of protocol deviations. These will be listed as a separate appendix, rather than in the body of the report. CMA status will be included in the listings.

6. Validation and QC

The following will be done by a second statistician: separate programming and checking of primary outcome results and conclusions; the statistical report will be read and sense-checked.

7. Data sharing

A file, or set of files, containing the final data will be prepared, along with a data dictionary. These will be made available to the Chief Investigator at the end of the analysis phase.

8. References

1. SAS® Institute Inc. SAS for Windows. SAS Institute Inc.: Cary, NC, U.S.A