The University Of Sheffield.

Sheffield Teaching Hospitals NHS Foundation Trust

Characterisation of a novel regimen of very low-dose aspirin combined with rivaroxaban in patients with chronic coronary syndromes:

WILL lOWer dose aspirin be better with rivaroxaban in patients with Chronic Coronary Syndromes? – (WILLOW CCS)

WILLOW

CCS

Clinical Study Protocol

RESEARCH REFERENCE NUMBERS

STH20412

TRIAL REGISTRY NUMBER

Clinicaltrials.gov: NCT04990791

OTHER RESEARCH REFERENCE NUMBERS

EudraCT: 2019-000267-25
MHRA CTA: 21304/0272/001-0001
REC Ref: 20/LO/0147
IRAS ID: 273020

SPONSOR
Sheffield Teaching Hospitals NHS Foundation Trust

This protocol has regard for the NHS Health Research Authority guidance and order of content

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and any subsequent amendments of the clinical trial regulations, GCP guidelines, the Sponsor's (and any other relevant) SOPs, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the trial publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the trial will be given; and that any discrepancies and serious breaches of GCP from the trial as planned in this protocol will be explained.

For and on behalf of the Trial Sponsor:

Signature: D Patel

Date: 17/08/2021

Name (please print):

Dipak Patel

Position:

Research & Innovation Manager

Chief Investigator:

Signature:

Date: ...../...../.....

Name: (please print): Professor Robert Storey

SIGNATURE PAGE

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For and on behalf of the Trial Sponsor:

Signature:

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Date:
...../...../.....

Name (please print):

.....

Position:

.....

Chief Investigator:

Signature:

.....

Date:
17/08/2021

Name: (please print): Professor Robert Storey

KEY TRIAL CONTACTS

Chief Investigator/Principal Investigator (Single site)	Professor Robert F. Storey Professor and Honorary Consultant in Cardiology Department of Infection, Immunity and Cardiovascular Disease University of Sheffield Beech Hill Road, Sheffield S10 2RX Telephone 0114 2261124 Fax 0114 3052008 Email r.f.storey@sheffield.ac.uk
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Sponsor	Sheffield Teaching Hospitals NHS Foundation Trust Clinical Research & Innovation Office D Floor, Royal Hallamshire Hospital Glossop Road, Sheffield S10 2JF Tel. 0114 2712763
Funder	Medical Research Council (MRC) Confidence in Concept Scheme
Clinical Research Facility	Sheffield Clinical Research Facility Centre for Biomedical Research Northern General Hospital Barnsley Road Drive, Sheffield S5 7AU
Pharmacy Co-ordinator	Mrs Kim Ryalls Pharmacy Department Northern General Hospital Herries Road, Sheffield S5 7AU

i. LIST of CONTENTS

ii. LIST OF ABBREVATIONS

AA	Arachidonic Acid
ACE	Angiotensin Converting Enzyme
ACS	Acute Coronary Syndrome
ADP	Adenosine Diphosphate
AE	Adverse Event
ANOVA	Analysis of Variance
APR	Annual Progress Report
AR	Adverse Reaction
AUC	Area Under the Curve
BD	Twice-daily
BHF	British Heart Foundation
BLT1	Leukotriene B receptor
BMI	Body Mass Index
CAD	Coronary artery disease
CCS	Chronic coronary syndromes
CI	Chief Investigator
COMPASS	Cardiovascular OutcoMes for People using Anticoagulation StrategieS
COX	Cyclo-oxygenase
CRF	Case Report Form
CRP	C-reactive Protein
CTA	Clinical Trial Authorisation
CYP	Cytochrome P450
DAPT	Dual Antiplatelet Therapy
DATT	Dual Antithrombotic Therapy
DSUR	Development safety update report
EP	Prostaglandin E receptor
EU	European Union
EudraCT	European Clinical Trials Database
GCP	Good Clinical Practice
GP	General Practitioner
HRA	Health Research Authority
ICF	Informed Consent Form
IHD	Ischaemic heart disease
IL-1	Interleukin 1
IL-6	Interleukin 6
IMP	Investigational Medicinal Product
IV	Intravenous
kg	Kilograms
L	Litres
LTB_4	Leukotriene B4
m^2	Metres Squared

MACE	Major Adverse Cardiovascular Events
mg	Milligrams
MHRA	Medicines and Healthcare products Regulatory Authority
MI	Myocardial Infarction
ml	Millilitres
mmHg	Millimetres of Mercury
ng	Nanograms
NGH-CRF	Northern General Hospital Clinical Research Facility
NHS	National Health Service
NIHR	National Institute for Healthcare Research
NIMP	Non-investigational medicinal product
NOAC	Non-vitamin K antagonist oral anticoagulant
NR	Not Recorded
NSAID	Non-Steroidal Anti-Inflammatory Drug
OD	Once-daily
$P2Y_{12}$	Platelet adenosine diphosphate receptor
PAD	Peripheral arterial disease
PCI	Percutaneous Coronary Intervention
PGI_2	Prostacyclin
PGI-M	Prostacyclin Metabolite
PI	Principal Investigator
PIS	Patient Information Sheet
PLATO	PLATelet inhibition and patient Outcomes
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
STH	Sheffield Teaching Hospitals
SUSAR	Suspected unexpected serious adverse reaction
TLR4	Toll-like Receptor 4
TMF	Trial Master File
TMG	Trial Management Group
TNF-α	Tumour Necrosis Factor α
TXA_2	Thromboxane A_2
TxM	Thromboxane metabolite
µg	Micrograms
UK	United Kingdom
µL	Microlitres
µmol	Micromoles
USA	United States of America

iii. TRIAL SUMMARY

Trial Title	WILL lOWer dose aspirin be better with rivaroxaban in patients with Chronic Coronary Syndromes? – (WILLOW CCS)	
Internal ref. no.	STH20412	
Clinical Phase	Phase IV	
Trial Design	Randomised controlled open-label crossover study	
Trial Participants	Men or women aged 18+ with a history of chronic coronary syndromes and high risk for atherothrombotic events, receiving antiplatelet monotherapy with aspirin 75 mg once daily	
Planned Sample Size	48	
Treatment duration	3 x 14 (-2) day periods	
Follow up duration	Until 14 (-2) days after last IMP dose	
Planned Trial Period	January 2020 – December 2020	
	Objectives	**Outcome Measures**
Primary	Assess effects on haemostasis	Bleeding time
Secondary	Assess fibrin clot dynamics	Lag time Lysis time Final clot turbidity
	Assess inflammatory parameters	Plasma interleukin 6 Plasma tumour necrosis factor α Serum C-reactive protein Circulating leukocyte count
	Assess effects on platelet function	Platelet aggregation responses to arachidonic acid, collagen and adenosine diphosphate
	Assess effects on key arachidonic acid metabolites	Serum thromboxane B_2 Urine prostacyclin metabolite Urine thromboxane metabolite
Investigational Medicinal Product(s)	1. Aspirin (aspirin lysine) 2. Rivaroxaban	
Formulation, Dose, Route of	1. 100 mg sachets for dissolution, administered orally as 20 mg twice-daily, or 75 mg once-daily	

Administration	2. 2.5 mg tablets administered orally twice-daily

iv. FUNDING AND SUPPORT IN KIND

FUNDERS	FINANCIAL AND NON FINANCIAL SUPPORT GIVEN
Medical Research Council (MRC)	Funding of the study (MRC Confidence in Concept Scheme)
Sheffield Teaching Hospitals NHS Foundation Trust (STHNFT)	Provision of space and services in kind to conduct the study

v. ROLE OF TRIAL SPONSOR AND FUNDER

The sponsor, STHNFT, will approve the protocol and associated study documents. The sponsor will contribute to site initiation, monitoring and close-out. They will also provide pharmacy support to the study.

The funder, MRC Confidence in Concept scheme, reviewed and approved an application for this study, and will provide monetary resources to complete it.

vi. ROLES AND RESPONSIBILITIES OF TRIAL MANAGEMENT COMMITTEES/GROUPS

The trial management group, chaired by the CI and including the sub-investigators, co-ordinators and laboratory team, will meet regularly to discuss a documented agenda. Minutes will be kept and shared with the sponsor. A trial management plan, agreed with the sponsor, will be written and a copy kept in the trial master file (TMF).

Given the small sample size, stable nature of the study population and exploratory nature of the study, in addition to the proven safety of the combination of aspirin and a low dose of factor-Xa inhibitor in the target population, there will not be a formal data monitoring committee. However, procedures defined in this protocol will be followed with regards to safety monitoring, including discussion with the sponsor in the event of any serious adverse event occurring.

vii. PROTOCOL CONTRIBUTORS

Dr William Parker led the writing of this protocol, under the supervision of the CI (Professor Rob Storey).

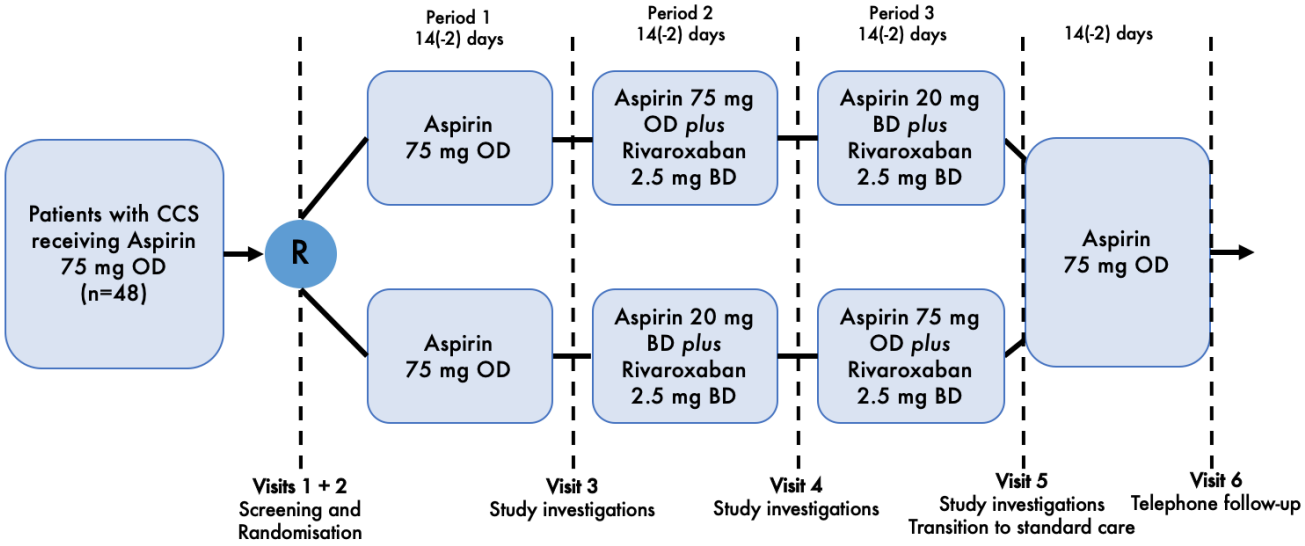
Dr Heather Judge assisted with designing laboratory methods.

Dr Erica Wallis critically reviewed the protocol on behalf of the sponsor.

Kim Ryalls and Mark Davy provided input into the pharmacy arrangements detailed in the protocol

viii. KEY WORDS: Aspirin, rivaroxaban, fibrin clot dynamics, inflammation, prostanoids, platelets, haemostasis

ix. TRIAL FLOW CHART



Key:
CCS – Chronic coronary syndrome
BD – Twice daily
LTA – Light transmittance aggregometry
OD – Once daily
PGI-M – Prostacyclin metabolite
R – Randomisation
TXB_2 – Thromboxane B_2
TxM – Thromboxane metabolite

Study Investigations

Pre-dose	Post-dose
Fibrin clot dynamics	Fibrin clot dynamics
Cytokines	Cytokines
Platelet function (LTA)	Platelet function (LTA)
Serum TXB_2	Serum TXB_2
Urine PGI-M & TxM	Urine PGI-M & TxM
Bleeding time	Bleeding time

1 BACKGROUND

The current standard antiplatelet regimen for secondary prevention of cardiovascular events for most patients with chronic coronary syndromes (CCS), including those with a history of stable angina or acute coronary syndromes >1 year ago, is aspirin 75 mg once-daily (OD) [1, 2]. The Cardiovascular OutcoMes for People using Anticoagulation StrategieS (COMPASS) study showed that, in high-risk individuals, adding rivaroxaban, a non-vitamin-K-antagonist oral anticoagulant (NOAC), at a dose of 2.5 mg twice-daily (BD) to standard aspirin treatment leads to a significantly lower risk of major adverse cardiovascular events (MACE) [3]. This may be because this combination, known as dual antithrombotic therapy (DATT), provides not only antiplatelet effects but also anticoagulation. Anticoagulation might affect parameters of fibrin clot dynamics that are an emerging risk factor for MACE [4, 5]. However, this approach also leads to increased bleeding [3], which dissuades clinicians and patients alike from long-term use. Whilst this may apply broadly across patient groups, there may be those in whom the risk-benefit profile is particularly difficult to balance, for example those over the age of 75 years or those with a history of CCS but at the lower end of the ischaemic-risk spectrum. Accordingly, those patients with CCS in whom DATT is currently not recommended may hypothetically derive benefit, over standard aspirin monotherapy, from a combination regimen with a better balance of anti-ischaemic benefit and harm from bleeding. Improving the safety profile of combination therapy with aspirin and a NOAC could lead to wider applicability of the approach and substantial improvement in clinical outcomes for patients at significant risk of ischaemic events.

Aspirin is a non-selective, irreversible inhibitor of cyclooxygenase (COX) 1 and 2 enzymes, at lower doses exhibiting relative pharmacokinetic selectivity for platelet COX1 [6], which is responsible for the synthesis of thromboxane A_2 (TXA_2), a pro-aggregant and vasoconstrictive prostanoid. At doses >325 mg/daily, based on the higher systemic concentration, aspirin can also significantly inhibit endothelial-derived COX2, leading to reduced release of the antithrombotic and vasodilatory compound prostacyclin (PGI_2). Aspirin is able to act as an antiplatelet drug by inhibiting TXA_2-mediated platelet activation without concurrent inhibition of PGI_2 synthesis entirely counteracting this [7]. Aspirin may also have beneficial effects on fibrin clot structure [8], although it is unclear if these translate into detectable differences in vivo.

We previously showed that a novel regimen of aspirin, 20 mg twice-daily (BD), reduced steady-state peak effects of aspirin, resulting in improved haemostasis, measured as bleeding time, compared to standard doses, in patients also receiving a second antithrombotic drug, ticagrelor [9]. Trough steady-state effects were maintained with adequate antiplatelet effect over a 24-hour period. We also saw a biologically-plausible reduction in pro-inflammatory cytokines with the novel regimen and, importantly, no differences in fibrin clot dynamics.

2 RATIONALE

Any antithrombotic regimen that provides a degree of anticoagulation and maintains adequate antiplatelet effects but avoids excess bleeding risk is highly attractive. A novel regimen of very-low-dose twice-daily aspirin (20 mg) and low-dose (2.5 mg) twice-daily rivaroxaban may have a range of benefits over standard aspirin therapy.

First, when compared to the standard regimen of DATT, it may provide a smoother profile of antithrombotic effect, with reduced peak-trough variation and hence reduced effects on haemostasis, mitigating the addition of a second agent. Second, when compared to standard aspirin therapy alone, the novel regimen may provide a degree of anticoagulation, for example increasing lag time and reducing lysis time, an independent predictor of MACE in patients with ischaemic heart disease (IHD) [10]. Third, as chronic aspirin treatment can potentiate the intravascular inflammatory response [11], when compared to both standard aspirin alone and DATT, a lower total dose might reduce any harmful proinflammatory effects, which might also improve fibrin clot dynamics further [12]. Targeting inflammation is an emerging effective strategy for reducing cardiovascular risk [13]. Finally, using lower-dose aspirin might result in reduced harmful inhibition of COX2.

A combination of standard dose aspirin (75 mg OD) and a NOAC, rivaroxaban 2.5 mg BD, is licensed for the secondary prevention of IHD in patients at high risk of recurrent thrombotic events, for example those with multi-vessel coronary artery disease (CAD), history of myocardial infarction (requiring additional risk factors if <65 years old) or symptomatic peripheral arterial disease (PAD). However, this combination leads to significantly increased bleeding when compared to aspirin alone, and is not recommended for long-term use in patient groups in whom the risk of ischaemia and bleeding are more finely balanced. For example, whilst patients >75 years old at high-risk of ischaemia were included in the COMPASS study, the benefit of DATT vs. aspirin alone appeared more modest than in other age groups, although there was not a statistically-significant interaction between age group and efficacy of DATT [3].

However, if a combination of aspirin and a NOAC with a better safety profile were to be available, as well as offering potential benefits to those in whom DATT is already considered an option, this may expand the group that could benefit from DATT, including those patients who are not currently regarded as good candidates for DATT, maximising the benefits whilst minimising harm.

2.1 Assessment and management of risk

Aspirin and rivaroxaban are both widely used as antithrombotic drugs for the treatment of cardiovascular disease. Combining aspirin and rivaroxaban increases the risk of bleeding over aspirin alone [3], but is licensed for use in patients at high ischaemic risk.

Whilst we hypothesise there are advantages of the novel regimen over standard aspirin therapy and standard DATT, we will limit participants' exposure to the shortest time possible to maintain scientific integrity. Those receiving drugs that significantly interact with aspirin and/or rivaroxaban will not proceed to randomisation.

Known side effect profiles

Aspirin (Aspirin lysine)

Reported side effects of aspirin (aspirin lysine), taken from the current SmPC, include:

More common:

- Increased bleeding tendency (e.g. nose bleed, bleeding gums, bruises, intestinal bleeds and bleeding due to trauma). It is normal to note increased bleeding with cuts or spontaneous bruising with any dose of aspirin.

- Stomach irritation. This is usually mild and, if necessary, can often be treated with acid-lowering medications. There is some evidence that lower doses of aspirin have a lower chance of causing irritation than high doses.

Less common side effects of aspirin:

- Rhinitis, shortness of breath and itching have been reported uncommonly (up to 1 in 100 patients).
- Blood count abnormalities, allergic reactions, bleeding into the brain, inflammation of the blood vessels, asthma, heavy periods, nausea and vomiting have been reported rarely (up to 1 in 1000).
- Other cases of renal failure, water retention, stomach or intestinal ulceration, headache, ringing in the ears, reduced hearing, vertigo and diarrhoea have been reported (exact chance not known).

Rivaroxaban

Reported side effects of rivaroxaban (at doses up to 20 mg once daily) include the following, reproduced from the current SmPC (DVT, deep vein thrombosis; NVAF, non-valvular atrial fibrillation; PE, pulmonary embolism; VTE, venous thromboembolism) [14]:

Common	Uncommon	Rare	Very rare	Not known
Blood and lymphatic system disorders				
Anaemia (incl. respective laboratory parameters)	Thrombocytosis (incl. platelet count increased)[A], Thrombocytopenia			
Immune system disorders				
	Allergic reaction, dermatitis allergic, Angioedema and allergic oedema		Anaphylactic reactions including anaphylactic shock	
Nervous system disorders				
Dizziness, headache	Cerebral and intracranial haemorrhage, syncope			
Eye disorders				
Eye haemorrhage (incl. conjunctival haemorrhage)				
Cardiac disorders				
	Tachycardia			

Vascular disorders				
Hypotension, haematoma				
Respiratory, thoracic and mediastinal disorders				
Epistaxis, haemoptysis				
Gastrointestinal disorders				
Gingival bleeding, gastrointestinal tract haemorrhage (incl. rectal haemorrhage), gastrointestinal and abdominal pains, dyspepsia, nausea, constipation[A], diarrhoea, vomiting[A]	Dry mouth			
Hepatobiliary disorders				
Increase in transaminases	Hepatic impairment, Increased bilirubin, increased blood alkaline phosphatase[A], increased GGT[A]	Jaundice, Bilirubin conjugated increased (with or without concomitant increase of ALT), Cholestasis, Hepatitis (incl. hepatocellular injury)		
Skin and subcutaneous tissue disorders				
Pruritus (incl. uncommon cases of generalised pruritus), rash, ecchymosis, cutaneous and subcutaneous haemorrhage	Urticaria		Stevens-Johnson syndrome/ Toxic Epidermal Necrolysis , DRESS syndrome	
Musculoskeletal and connective tissue disorders				
Pain in extremity[A]	Haemarthrosis	Muscle haemorrhage		Compartment syndrome secondary to a bleeding
Renal and urinary disorders				
Urogenital tract haemorrhage (incl. haematuria and				Renal failure/acute renal failure secondary to a bleeding sufficient

menorrhagia[B]), renal impairment (incl. blood creatinine increased, blood urea increased)				to cause hypoperfusion
General disorders and administration site conditions				
Fever[A], peripheral oedema, decreased general strength and energy (incl. fatigue and asthenia)	Feeling unwell (incl. malaise)	Localised oedema[A]		
Investigations				
	Increased LDH[A], increased lipase[A], increased amylase[A]			
Injury, poisoning and procedural complications				
Postprocedural haemorrhage (incl. postoperative anaemia, and wound haemorrhage), contusion, wound secretion[A]		Vascular pseudoaneurysm[C]		
A: observed in prevention of VTE in adult patients undergoing elective hip or knee replacement surgery B: observed in treatment of DVT, PE and prevention of recurrence as very common in women < 55 years C: observed as uncommon in prevention of atherothrombotic events in patients after an ACS (following percutaneous coronary intervention)				

Mitigation of risks

Any risks in this study are mitigated by:

- a robust informed consent procedure, followed before any study-related activities take place
- ensuring at screening that potential participants do not have any medical or medication issues that would preclude safe involvement in the study
- excluding those with co-morbidities or receiving concomitant medication likely to significantly increase the risk of taking part in the study
- as short as feasible exposure to study drugs whilst maintaining scientific credibility
- rigorous safety monitoring by the study team and sponsor

3 OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

3.1 Primary objective

The primary objective of this study is to assess, in patients with a history of IHD currently receiving aspirin 75 mg once daily as antithrombotic monotherapy, the effects of a novel regimen of very-low-dose twice-daily aspirin (aspirin lysine) (20 mg BD) and rivaroxaban (2.5 mg BD), compared to a standard regimen of aspirin (aspirin lysine) monotherapy (75 mg OD), and a standard regimen of DATT (aspirin 75 mg OD plus rivaroxaban 2.5 mg BD) on post-dose bleeding time.

It is hypothesised that the difference in bleeding time when receiving aspirin (aspirin lysine) 75 mg once-daily alone vs. aspirin (aspirin lysine) 20 mg twice-daily plus rivaroxaban 2.5 mg twice-daily will be less than the difference in bleeding time between aspirin (aspirin lysine) 75 mg once-daily alone vs. aspirin (aspirin lysine) 75 mg once-daily plus rivaroxaban 2.5 mg twice-daily.

3.2 Secondary objectives

The secondary objectives of this study are to assess, in patients with a history of CCS, the effects of a novel regimen of very-low-dose twice-daily aspirin (aspirin lysine) (20 mg BD) and rivaroxaban (2.5 mg BD) compared to (i) a standard regimen of aspirin (aspirin lysine) monotherapy (75 mg OD) and (ii) a standard regimen of DATT (aspirin 75 mg OD plus rivaroxaban 2.5 mg BD) on:

1. Fibrin clot dynamics (lag time, lysis time, final clot turbidity)
2. Inflammatory parameters (plasma IL-6, TNFα; serum CRP; circulating leukocyte count)
3. Platelet function (platelet aggregation responses to arachidonic acid, collagen and adenosine diphosphate)
4. Arachidonic acid metabolites (serum TXB_2, urine PGI-M, urine TxM)

3.3 Outcome measures/endpoints

Primary endpoint/outcome

The primary endpoint will be difference in bleeding time, measured at 2 hours post-dose, assessed between aspirin (aspirin lysine) 75 mg once-daily alone vs. aspirin (aspirin lysine) 20 mg twice-daily plus rivaroxaban 2.5 mg twice-daily and aspirin (aspirin lysine) 75 mg once-daily alone vs. aspirin (aspirin lysine) 75 mg once-daily plus rivaroxaban 2.5 mg twice-daily.

Secondary endpoints/outcomes

When receiving aspirin (aspirin lysine) 20 mg BD plus rivaroxaban, vs. aspirin (aspirin lysine) 75 mg OD, vs. aspirin (aspirin lysine) 75 mg OD plus rivaroxaban 2.5 mg BD:

1. Bleeding time pre-dose
2. Fibrin clot lag time pre-dose and 2 hours post-dose
3. Fibrin clot lysis time pre-dose and 2 hours post-dose
4. Plasma TNF-α pre-dose and 2 hours post-dose
5. Plasma IL-6 pre-dose and 2 hours post-dose
6. Serum CRP 2 hours post-dose
7. Leukocyte count pre-dose and 2 hours post-dose
8. Serum TXB_2 pre-dose and 2 hours post-dose
9. Urine PGI-M pre-dose and 2 hours post-dose
10. Urine TxM pre-dose and 2 hours post-dose

11. Mean platelet aggregation responses to arachidonic acid (AA), collagen and adenosine diphosphate (ADP), pre-dose and 2 hours post-dose
12. Final clot turbidity pre-dose and 2 hours post-dose

Secondary endpoints will be compared between the three groups by repeated-measures ANOVA (1,2) and paired t-tests (3-9), where appropriate, between:

1. Post-dose (aspirin 20 mg BD plus rivaroxaban 2.5 mg BD) vs. post-dose (aspirin 75 mg OD) vs. post-dose (aspirin 75 mg OD plus rivaroxaban 2.5 mg BD)
2. Pre-dose (aspirin 20 mg BD plus rivaroxaban 2.5 mg BD) vs. pre-dose (aspirin 75 mg OD) vs. pre-dose (aspirin 75 mg OD plus rivaroxaban 2.5 mg BD)
3. Pre-dose (aspirin 20 mg BD plus rivaroxaban 2.5 mg BD) vs. post-dose (aspirin 20 mg BD plus rivaroxaban 2.5 mg BD)
4. Pre-dose (aspirin 75 mg OD) vs. post-dose (aspirin 75 mg OD)
5. Pre-dose (aspirin 75 mg OD plus rivaroxaban 2.5 mg BD) vs. post-dose (aspirin 75 mg OD plus rivaroxaban 2.5 mg BD)
6. Post-dose (aspirin 20 mg BD plus rivaroxaban 2.5 mg BD) vs. post-dose (aspirin 75 mg OD)
7. Post-dose (aspirin 20 mg BD plus rivaroxaban 2.5 mg BD) vs. post-dose (aspirin 75 mg OD plus rivaroxaban 2.5 mg BD)
8. Pre-dose (aspirin 20 mg BD plus rivaroxaban 2.5 mg BD) vs. pre-dose (aspirin 75 mg OD)
9. Pre-dose (aspirin 20 mg BD plus rivaroxaban 2.5 mg BD) vs. pre-dose (aspirin 75 mg OD plus rivaroxaban 2.5 mg BD)

4 TRIAL DESIGN

The trial is a pharmacodynamic study to determine the effect of a novel regimen of aspirin 20 mg BD plus rivaroxaban 2.5 mg BD on haemostasis, fibrin clot dynamics, inflammatory markers, platelet function and arachidonic acid metabolites when compared to standard regimens of aspirin 75 mg OD and aspirin 75 mg OD plus rivaroxaban 2.5 mg BD.

In a randomised open-label three-period crossover design, patient participants receiving aspirin 75 mg OD for secondary prevention of IHD will be randomised 1:1 to receive one of two sequences of aspirin: aspirin 75 mg OD, then aspirin 20 mg BD plus rivaroxaban 2.5 mg BD, then aspirin 75 mg OD plus rivaroxaban 2.5 mg BD; or aspirin 75 mg OD, then aspirin 75 mg OD plus rivaroxaban 2.5 mg BD, then aspirin 20 mg BD plus rivaroxaban 2.5 mg BD.

This design allows comparison of the three regimens with a lead-in period on the baseline antiplatelet treatment participants are receiving at enrolment, but replaced with a soluble aspirin preparation to allow accurate comparisons with the other treatments. Having the two periods including rivaroxaban adjacent to each other, but in a randomised order, allows simplification of dispensing arrangements allowing us to maximise use of resources available to conduct the study, and removes any concern over carryover effects of rivaroxaban relevant to study measurements into a period of aspirin monotherapy.

At the end of each 14(-2) day medication period, they will attend a study visit at which blood and urine samples will be obtained, and bleeding time measured, before and 2 hours after the last dose of IMP of the treatment period. The samples will be tested for fibrin clot dynamics; inflammatory markers and cytokines; prostanoids; and platelet function.

Participants will be transitioned back to standard-of-care aspirin 75 mg OD at the end of the third treatment period and followed up by telephone call 14(-2) days later.

5 TRIAL SETTING

This will be a single-centre open-label study, conducted by the Cardiovascular Research Unit, University of Sheffield within the Clinical Research Facility at the Northern General Hospital, Sheffield.

Participants will be outpatients with a history of ischaemic heart disease currently receiving aspirin 75 mg OD, recruited from the local area.

There is anticipated to be a large population of eligible potential participants under the current or previous care of the Cardiology and Cardiothoracic Surgery Directorate of Sheffield Teaching Hospitals NHS Foundation Trust, meaning a single-centre design is appropriate without the need for any separate participant identification centres.

6 PARTICIPANT ELIGIBILITY CRITERIA

6.1 Inclusion criteria

To participate in this trial, subjects must meet all of the following criteria:

For inclusion in the study, subjects should fulfil the following criteria:

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

2. Male or female aged greater than 18 years

3. Existing diagnosis of a chronic coronary syndrome:

   (i) History of stable angina

   or

   (ii) History of an acute coronary syndrome event >1 year ago

   or

   (iii) Previous evidence on imaging of either at least one stenosis >50% in an epicardial coronary artery or a myocardial perfusion defect

4. High-risk for atherothrombotic events, defined as:

   (i) aged 65 years or over

   or

   (ii) atherosclerosis in at least 2 vascular territories (such as coronary, cerebrovascular, or peripheral arteries)

   or

(iii) Two or more of the following: current smoking, diabetes mellitus, estimated glomerular filtration rate (eGFR) less than 60 ml/min, heart failure, previous non-lacunar ischaemic stroke.

5. Receiving single antiplatelet therapy with aspirin 75 mg once daily

6.2 Exclusion criteria

Subjects meeting any of the following criteria will be excluded from participation in the study:

1. Any history of haemorrhagic stroke or lacunar stroke
2. History of ischaemic stroke or transient ischaemic attack in the last year
3. Heart failure associated with NYHA class III or IV symptoms
4. Estimated glomerular filtration rate <30 ml/min
5. Planned procedure for coronary revascularization
6. Any planned surgery or other procedure that may require suspension or discontinuation of antiplatelet therapy expected to occur within 3 months of randomisation
7. Prior intention by patient or physician to discontinue aspirin within the study period
8. Receiving doses of aspirin other than 75 mg once daily
9. Treatment or planned treatment with antiplatelet medication apart from aspirin (eg. clopidogrel, prasugrel, ticagrelor, dipyridamole, ticlopidine)
10. Current use of a loop, thiazide or potassium sparing diuretic (affects prostanoid assays)
11. Any acute coronary syndrome event, percutaneous coronary intervention or coronary artery bypass grafting within 1 year prior to randomisation
12. Current or planned use of an oral anticoagulant (e.g. warfarin, dabigatran, rivaroxaban [including 2.5 mg BD], apixaban, edoxaban) or parenteral anticoagulant (eg. unfractionated heparin, low molecular weight heparin, bivalirudin)
13. Current or planned use of a GPIIb/IIIa inhibitor (eg. abciximab, tirofiban)
14. Current or planned use of a fibrinolytic agent (eg. tissue plasminogen activator)
15. Requiring or likely to require treatment with a non-steroidal anti-inflammatory drug (NSAID), including COX2 inhibitors, and including regular or intermittent/as required use
16. Current or planned use of a strong CYP3A4 inhibitor (eg, ketoconazole, itraconazole, voriconazole, telithromycin, clarithromycin [but not erythromycin or azithromycin], nefazadone,

ritonavir, saquinavir, nelfinavir, indinavir, atanazavir, cobicistat or over 1 litre daily of grapefruit juice), strong inducer (e.g. rifampin/rifampicin, rifabutin, phenytoin, carbamazepine, phenobarbital), a selective serotonin reuptake inhibitor (SSRI) or selective noradrenergic reuptake inhibitor (SNRI).

17. Current or planned use of the following: methotrexate at a dose >15 mg per week; tenofovir; or a uricosuric drug (e.g. probenecid, benzbromarone and sulfinpyrazone).

18. Clinically significant liver disease, defined as known or suspected diagnosis of hepatic cirrhosis with current Child Pugh class B or C; or elevation of serum alanine transferase or aspartate transferase greater than 3 times the upper limit of the normal range for the processing laboratory.

19. History of alcohol or drug abuse, defined as regular use of an illicit substance for recreational purposes or regular consumption of greater than 50 units (males) or 35 units (females) of alcohol per week, in the last year

20. Co-morbidity associated with life expectancy less than 1 year

21. Any other condition deemed by the investigator to significantly affect haemostasis, coagulation, bleeding risk or ability to comply with the study protocol.

22. Women of child-bearing potential (WOCBP)[A] unless negative pregnancy test at screening and willing to use highly-effective contraception[B] for the duration of treatment with study medication.

23. Pregnant or breast-feeding women.

24. Any contraindication for aspirin (aspirin lysine) or rivaroxaban treatment as detailed in the respective SmPCs.

NOTES

A. WOCBP are defined as women who are fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.

B. Highly-effective methods of contraception are defined as combined (oestrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal or transdermal), progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable or implantable), intrauterine device, intrauterine hormone-releasing system, bilateral tubal occlusion, vasectomised partner or sexual abstinence. Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.

7 TRIAL PROCEDURES

Details of analysis of blood & urine samples

The aim of the analysis of the study samples will be to: (i) show the effects of aspirin 20 mg BD plus rivaroxaban 2.5 mg BD on, haemostasis, fibrin clot dynamics, prostanoids, platelet function and inflammation, when compared to standard aspirin 75 mg OD and aspirin 75 mg OD plus rivaroxaban 2.5 mg BD.

Haemostasis will be assessed by measuring, in steady state, bleeding time pre- and 2 hours post-dose using a method shown to be sensitive to additive effects of antiplatelet agents [15]. The procedure for measuring bleeding time is described in the study-specific SOP relating to this.

We will measure parameters of fibrin clot formation and lysis, specifically lag time, final clot turbidity and lysis time.

To determine the detailed effects of the three regimens on prostanoid synthesis, we will measure the principal metabolites of TXA_2 and PGI_2 (serum TXB_2 and urinary PGI-M respectively).

Effects on the inflammatory state will be assessed by measurement of the inflammatory markers TNF-α, IL-6 and CRP.

Finally, in order to confirm the efficacy of the antiplatelet aspects of the drug regimens being studied, the activity of a broad range of pathways of platelet activation and aggregation will be assessed by light transmittance aggregometry, pre-medication and 2 hours post-medication, using arachidonic acid 0.3 and 1 mmol/L, collagen 4 and 16 µg/mL, and ADP 20 µmol/L as agonists.

Study samples will either be measured immediately after collection (e.g. light transmittance aggregometry) or stored for assays at a later time (other endpoints).

We will also store acellular samples of serum, plasma, deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and urine within the Cardiovascular Research Unit for future, as yet unplanned, studies. Consent will be sought for this at study enrolment.

7.1 Recruitment

7.1.1 Participant identification

Potential participants will be identified through review of the records of the South Yorkshire Cardiothoracic Centre and/or by referral by their clinical team.

Specifically, participants may be approached in the following ways:

1. An invitation letter sent by post in combination with a copy of the participant information sheet. There will be a reply slip which they can return by post, and there will be contact details (telephone and email) for the research team to allow them to respond by these methods too.

2. Directly by telephone. In this case, should they be happy to learn more, they will be sent a copy of the invitation letter, participant information sheet and reply slip by post or email, depending on their preference. If they agree to attend for study screening, they will communicate this to the research team by returning the reply slip by post or email, or by contacting the research team directly using the contact details provided to them.
3. Directly in clinics within the Cardiology and Cardiothoracic Surgery Directorate at Sheffield Teaching Hospitals NHS Foundation Trust, upon referral from their clinical team. In this case they will be provided with a copy of the participant information sheet and, if unable to decide whether they wish to attend for screening or not during the clinic visit, will be signposted to the contact details of the research team to provide their response.

Potential participants who are interested in taking part in the study will then be contacted by the research team to book a screening appointment.

7.1.2 Screening

Screening will occur at visit 1. The following study procedures will be performed, after obtaining written consent for the study:

- Medical history
- Physical examination
- Collection of demographic data
- Vital signs (pulse, blood pressure and temperature).
- Height, weight and BMI
- Recording of any concomitant medication
- Safety blood tests (13.5 ml blood sample for haematology, clinical chemistry [including serum pregnancy test in WOCBP] and coagulation)
- Urinalysis (dipstick)
- Baseline electrocardiogram

7.1.3 Payment

Volunteers will receive no payment for taking part in the study, however travel costs to and from the Clinical Research Facility will be reimbursed or transportation by taxi arranged.

7.2 Consent

Written, informed consent, using the current version of the approved designated form for this study, will be obtained prior to any study procedures being carried out. This will be explained and obtained by a medically-qualified member of the research team, listed on the delegation log. Participants will have the chance to read the ICF/PIS for as long as they need, and will be able to ask any questions, prior to signing.

Minors and those judged to be without the mental capacity to provide informed consent will not be enrolled into the study.

Participants will remain free to withdraw at any time from the trial, without giving reasons and without prejudicing his/her further treatment, and will be provided with a contact point where he/she may obtain further information about the trial. Samples collected up to the point of withdrawal will only be used after withdrawal if the participant consents for this, otherwise they will be destroyed. However, data collected up to that point will be used for analysis, and this will be explicitly stated in the participant information sheet and consent form.

7.2.1 Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable

As described above, we will also store acellular samples of serum, plasma, DNA, RNA and urine for future, as yet unplanned, studies. Explicit consent for this will be sought on the consent form at enrolment. Any cellular samples taken will be destroyed before the end of the study.

7.3 The randomisation scheme

Participants will be randomised to one of the following two treatment sequences, in a 1:1 fashion:

(A) Aspirin 75 mg OD for 14(-2) days *then* aspirin 20 mg BD plus rivaroxaban 2.5 mg BD for 14(-2) days *then* aspirin 75 mg OD plus rivaroxaban 2.5 mg BD

or

(B) Aspirin 75 mg OD for 14(-2) days *then* aspirin 75 mg OD plus rivaroxaban 2.5 mg BD for 14(-2) days *then* aspirin 20 mg BD plus rivaroxaban 2.5 mg BD

7.3.1 Method of implementing the randomisation/allocation sequence

Randomisation will be handled by an online interactive web-based randomisation service, sealedenvelope.com. Their 'simple randomisation service' will be used and a study-specific SOP will be prepared and followed.

Participants will be allocated a three-digit number at enrolment (starting at 001) prefixed with 'E01' (e.g. E01001), then if they proceed to randomisation they will be allocated a separate three-digit randomisation number (starting at 001) prefixed with 'R' (e.g. R001).

The system will generate an immediate email to the investigators stating the treatment allocation and this will be printed, one copy being placed in the participant's study file and a further copy sent to the Northern General Hospital Pharmacy with a study-specific prescription for study medication during period 1. A separate prescription will be issued to cover periods 2 and 3 but using the same randomisation document as evidence of the allocation.

7.4 Blinding

This study will be open-label i.e. unblinded to participants and investigators throughout. However, those performing the laboratory assessments will be blinded to treatment allocation in order to reduce bias.

7.5 Emergency Unblinding

Procedures for emergency unblinding are not relevant to this study.

7.6 Baseline data

At visit 1

- Medical history
- Physical examination
- Demographic data
- Vital signs (pulse, blood pressure and temperature)
- Weight and BMI
- Concomitant medication
- Lab safety parameters (full blood count, urea & electrolytes, liver function tests, clotting screen, dipstick urinalysis, urinary pregnancy test if female and of childbearing potential)
- Electrocardiogram findings

At visit 2

- Vital signs: pulse, blood pressure and temperature
- Physical examination

7.7 Trial assessments

Visit 1 - Screening (Day -21 to 0)

Screening of subjects and all study-related procedures will take place in the Sheffield Clinical Research Facility, a specialist environment for the conduct of clinical research. The following assessments and procedures will be performed:

- Full informed consent, including completion of the informed consent form

- Inclusion/exclusion criteria (see section 6)
- Medical history
- Physical examination
- Demographic data
- Vital signs: pulse, blood pressure and temperature
- Weight and BMI
- Concomitant medication
- Lab safety (13.5 ml blood sample for haematology; clinical chemistry & coagulation; urinalysis)
- Electrocardiogram

If eligible, subjects will be asked to return to the Clinical Research Facility, Northern General Hospital, on Day 0 for the experimental part of the study. They will be randomised to receive a three-period medication regimen as detailed in section 7.3 of this protocol. Providing results of all screening tests are available before visit 2 is commenced, visit 2 may occur on the same day as visit 1.

A letter will be sent to the participant's GP to inform them of enrolment in the study. Transportation to and from the Clinical Research Facility will be provided if necessary. Subjects who fail screening will be recorded on a screen failure log with the reason for failure.

Visit 2 (Day 0) - Randomisation

- Vital signs
- Physical examination
- Adverse event recording
- Reconfirm eligibility criteria met (by a medically qualified member of the study team, see section 6) and no withdrawal criteria met (section 7.10)
- Randomisation
- Provided with supply of aspirin (aspirin lysine) for periods 1, 2 and 3 (2 boxes of 30 sachets)
- Dose-preparation training for aspirin (aspirin lysine) 75 mg OD, including supply of written illustrated instructions
- Issue with participant information card detailing treatment allocation, restrictions during the study and contact details for the research team

Period 1: 14 (-2) days

- Participants will receive aspirin (aspirin lysine) 75 mg OD, but should withhold their dose on the morning of visit 3 (during which the dose will be taken).

Visit 3 - Period 1: Day 14 (-2)

- Vital signs
- Physical examination
- Adverse event recording
- Concomitant medication recorded
- Venous blood sample pre- and 2 hours post-dose for fibrin clot dynamics, inflammatory markers, prostanoids and platelet function
- Bleeding time pre- and 2 hours post dose
- Urine sample pre- and 2 hours post-dose for prostanoids
- IMP compliance check for period 1
- Dispensing of rivaroxaban 2.5 mg tablets for periods 2 and 3 (total 56 tablets)
- Urine pregnancy test for women of childbearing potential (must not continue to period 2 if positive)
- Dose-preparation training for aspirin (aspirin lysine) 20 mg BD if allocated to sequence A, including provision of written instructions

Period 2: 14(-2) days

Participants will receive their allocated regimen for period 2 for 14(-2) days:

  - If randomised to sequence A they will receive aspirin (aspirin lysine) 20 mg BD plus rivaroxaban 2.5 mg BD in period 2.
  - If randomised to sequence B they will receive aspirin (aspirin lysine) 75 mg BD plus rivaroxaban 2.5 mg BD in period 2.

Participants should withhold their dose on the morning of visit 4 (during which the dose will be taken).

Visit 4 : 14(-2) days into period 2

- Vital signs
- Physical examination
- Adverse event recording

- Concomitant medication recorded
- Venous blood sample pre- and 2 hours post-dose for fibrin clot dynamics, inflammatory markers, prostanoids and platelet function
- Bleeding time pre- and 2 hours post dose
- Urine sample pre- and 2 hours post-dose for prostanoids
- Urine preganacy test for WOCBP
- IMP compliance recorded for period 2
- Dose-preparation training for aspirin (aspirin lysine) 75 mg OD if allocated to sequence A or 20 mg BD if sequence B, including provision of written instructions

Period 3: 14(-2) days

Participants will receive their allocated regimen for period 3 for 14(-2) days:

  - If randomised to sequence A they will receive aspirin (aspirin lysine) 75 mg OD plus rivaroxaban 2.5 mg BD in period 3.
  - If randomised to sequence B they will receive aspirin (aspirin lysine) 20 mg BD plus rivaroxaban 2.5 mg BD in period 3.

Participants should withhold their dose on the morning of visit 5 (during which the dose will be taken).

Visit 5 : 14(-2) days into period 3

- Vital signs
- Physical examination
- Adverse event recording
- Concomitant medication recorded
- Venous blood sample pre- and 2 hours post-dose for fibrin clot dynamics, inflammatory markers, prostanoids and platelet function
- Bleeding time pre- and 2 hours post dose
- Urine sample pre- and 2 hours post-dose for prostanoids
- Urine pregnancy test for WOCBP
- IMP compliance recorded for period 3

- Collect and return unused medication to pharmacy
- Transition to standard of care aspirin 75 mg OD, ensuring participant has a supply of this

Visit 6 : 14(-2) days after visit 5 (Telephone call)

- Telephone follow-up for adverse events and concomitant medication

7.8 Long term follow-up assessments

Participants will be followed until 14(-2) days after visit 5 by telephone. Any new adverse events will be recorded. Participants will be thanked for their involvement in the study. In the event of a participant remaining uncontactable at the end of the follow-up window, we will interrogate the hospital records of the participant to clarify vital status and any evidence of untoward events. Participants will be declared 'lost to follow up' if they are uncontactable 28 days after visit 5. A file note will be made in this instance.

7.9 Qualitative assessments

Not applicable to this study

7.10 Withdrawal or postponement criteria

Subjects may be withdrawn or may discontinue from the trial if the following occur:

- Inability to obtain venous blood samples
- Withdrawal of consent
- Development of an intolerable adverse event due to study participation as determined by the investigator and/or subject
- Development of a significant intercurrent illness, condition or procedural complication that would interfere with the subject's continued participation, unless further study activities can be appropriately postponed (see section 7.10.1)
- Participant becomes aware of pregnancy or positive pregnancy test at visit 3
- At the time of planned study visit 3, 4 or 5, receipt of a non-study medication in the preceding 48 hours that, in the opinion of the investigator, is likely to significantly affect thrombosis, haemostasis, inflammation or prostanoid synthesis, unless the visit can be appropriately postponed (see section 7.10.1 and section 8.9)
- Violation of the protocol

- The investigator feels it is medically in the best interest of the subject to discontinue the subject's participation in the study
- Previously unknown data becoming available raising concern about the safety of the study drugs, so that continuation could cause potential risks to the subjects

The reason for withdrawal/discontinuation will be documented in the Case Report Form (CRF).

Withdrawn participants who receive at least one dose of IMP will be followed up by telephone at 14(-2) days after withdrawal, and additionally all who discontinue due to adverse events (AEs) will be followed up until resolution or stabilisation. All outcomes of AEs will be recorded in the CRF.

Participants withdrawn before visit 5 is completed will be replaced in order to maintain statistical power.

7.10.1 Provision for postponement of visit in the event of intercurrent illness

In the event of the following occurring in relation to a study participant:

- a significant intercurrent illness, for example a lower respiratory tract infection requiring antibiotic therapy;
- requirement for medication that, in the opinion of the investigators, is likely to affect measures of inflammation, thrombosis, haemostasis or prostanoid synthesis (see section 8.9);

then if the subsequent study visit 3 or 4 cannot be postponed to a time within the permitted window of 14(-2) days into the medication period at which the illness or relevant effect of medication has deemed to have resolved, study medication may be stopped (and transitioned to standard of care aspirin 75 mg OD) and the medication period may be restarted once the intercurrent illness or requirement for medication is deemed to have resolved. In this instance, an unscheduled visit will be arranged, the existing supply of aspirin (aspirin lysine) +/- rivaroxaban will be counted and, if necessary, a further supply of aspirin (aspirin lysine) +/- rivaroxaban will be issued, dispensed after study-specific prescription request has been made to the pharmacy at the Northern General Hospital. A file note will be made in the TMF.

7.11 Storage and analysis of clinical samples

The samples will be stored and analysed according to the study-specific laboratory manual. Samples are anticipated to be analysed by the following laboratories and methods:

Cardiovascular Research Unit, University of Sheffield

1. Fibrin clot lysis time by fibrin clot turbidimetry [10]
2. Fibrin clot lag time by fibrin clot turbidimetry [10]
3. Fibrin clot final turbidity by fibrin clot turbidimetry [10]
4. Plasma TNF-α by enzyme-linked immunosorbent assay (ELISA)/multiplex bead assay (MBA) [16] [12]
5. Plasma IL-6 by ELISA/MBA [16] [12]
6. Leukocyte count (and subsets), by automated cell counting [17]
7. Serum TXB_2 by ELISA [18]
8. Urine PGI-M by ELISA [19]
9. Urine TxM by ELISA [20]

10. Platelet aggregation responses to AA, collagen and adenosine diphosphate ADP by light transmittance aggregometry [18]

Department of Laboratory Medicine, Sheffield Teaching Hospitals NHS Foundation Trust

1. Serum CRP (Roche Cobas assay) [21]
2. Urinary creatinine (required as a denominator for the correct analysis of urinary PGI-M and TxM) (Roche Cobas assay) [18]

7.12 End of study

The study will end when the last laboratory analyses are completed. The sponsor will notify the MHRA of the end of the study within 90 days of its completion. In the event of early termination, this will be reported within 15 days.

8 STUDY MEDICATIONS

8.1 Name and description of investigational medicinal product(s)

The following medications will be used for experimental purposes during the study:

- Aspirin lysine (e.g. 'Aspegic' [Sanofi-Aventis] '100 mg' sachets)
    - Each aspirin lysine '100 mg' sachet contains 180 mg aspirin lysine, equivalent to 100 mg acetylsalicylic acid (aspirin). In this protocol the stated dose refers to the aspirin content.
    - Participants will be asked to prepare oral doses of 20 mg twice daily, where directed by the protocol, by dissolving 100 mg (1 sachet) in 100 ml of drinking water and ingesting 20 ml of the solution using a graduated syringe, discarding the remainder.
    - Participants will be asked to prepare oral doses of 75 mg once daily, where directed by the protocol, by dissolving 100 mg (1 sachet) in 100 ml of drinking water, removing 25 ml of the solution using a graduated syringe, and ingesting the remainder.
    - A new sachet will be used for each dose of study medication
- Rivaroxaban 2.5 mg tablets for oral ingestion (Bayer, 56 tablets per pack)
    - When specified by the randomisation allocation, participants will be asked to take one 2.5 mg rivaroxaban tablet twice a day, in the morning and evening, commencing the morning after visits 3 and 4.

8.2 Regulatory status of the drug

- Aspirin (aspirin lysine) 100 mg sachets are not licensed in the UK but are licensed in other EU countries (e.g. Belgium, Italy) for the purposes of analgesia and antipyresis. Other preparations of aspirin are licensed in the UK for similar indications, as well as an antithrombotic agent after ACS.
- As there is no marketing authorisation in the UK for aspirin lysine, we will import this from another EU country facilitated by an existing relationship with Mawdsleys Ltd.

- Rivaroxaban at a dose of 2.5 mg BD is licensed, including in the UK, for the prevention of atherothrombotic events in adult patients with CAD or symptomatic PAD at high risk of ischaemic events, when taken in combination with aspirin 75-100 mg OD.
- We will retain the original presentation of drugs as supplied without modification, but will be asking participants to vary the regimens of aspirin (aspirin lysine) and rivaroxaban as detailed elsewhere in this protocol.

8.3 Product Characteristics

Aspirin (aspirin lysine) 100 mg sachets have a current summary of product characteristics (SmPC) that has been translated and notarised from the original French. A copy will be kept in the trial management file.

The current version of the SmPC for rivaroxaban 2.5 mg tablets will also be available for reference in the trial management file.

The study team will check for updates to these documents at regular intervals throughout the development and conduct of the study, and will review and file these as needed, if necessary obtaining further notarised translations. If an update to section 4.8 (the reference safety information) is involved, the revised SmPC will be submitted and approved as a substantial amendment before the revised SmPC is filed as the new reference safety information for the trial for the assessment of expectedness of adverse events.

8.4 Drug storage and supply

Aspirin (aspirin lysine) 100 mg sachets will be sourced by the Northern General Hospital Pharmacy through a study-specific arrangement with Mawdsleys Ltd at market price.

Rivaroxaban 2.5 mg tablets will be sourced by the Northern General Hospital Pharmacy locally at market price.

The initial shipment will be requested on site initiation (manual ordering) and re-ordering will occur manually as necessary until the end of the study.

Prior to study medication dispensing, all study medications will be kept in a secure location in the pharmacy at Northern General Hospital, Sheffield, under appropriate storage conditions with temperature excursions permitted between 15°C and 30°C. Aspirin (aspirin lysine) will be segregated as clinical trial stock for the purposes of this study. Rivaroxaban will be dispensed from unsegregated clinical pharmacy stock unless practical reasons for segregation of stock arise.

Aspirin (aspirin lysine) and rivaroxaban will be supplied to the investigators upon receipt of a suitable signed study-specific prescription at the start of periods 1 and 2 respectively. Only medically-qualified members of the study team will be able to complete the prescription. At the start of period 1, two boxes of 30 sachets of aspirin (aspirin lysine) will be dispensed for each participant. At the start of period 2, one box of 56 rivaroxaban 2.5 mg tablets will be dispensed for each participant.

There are no specific post-dispensing storage instructions.

Unused medication and used packaging will be returned to pharmacy at the end of treatment period 3. Pharmacy will perform pill/sachet count for accountability and will supervise destruction.

In the event of postponing visit 3, 4 or 5 because of intercurrent illness and restarting a medication period from the beginning, unused medication from the period prior to the postponement will be counted by the investigators and, if necessary to ensure adequate amounts to restart the period, a new study-specific prescription will be made by a medically-qualified investigator for aspirin (aspirin lysine) +/- rivaroxaban.

8.5 Preparation and labelling of Investigational Medicinal Product

Labels for aspirin (aspirin lysine) and rivaroxaban will be prepared in accordance with Good Manufacturing Practice and regulatory guidelines of the Medicines Healthcare and Regulatory Agency. The label will include the following information: drug, formulation, dose, and dosing frequency as well as other information to comply with annex 13.

Participants will also be given written, illustrated dose-preparation instructions for aspirin (aspirin lysine). They will be trained to prepare the correct dose of aspirin for periods 1 to 3 at visits 2 to 4.

8.6 Dosage schedules

The dosage schedules are described in section 7.3, 7.7 and the trial flow chart within this protocol.

When taking once-daily doses of study medication, this will be on waking, with the exception of the days of visits 3, 4 and 5 when they will be asked to delay taking until the appropriate point within the study visit. When taking twice-daily, this should be on waking (with the exception of the days of visits 4 and 5 when they will be asked to delay taking until the appropriate point within the study visit) and then as close to 12 hours later as possible.

In the case of a missed dose, the participant will be advised to take the dose when they remember if within 6 hours (twice-daily regimens) or 12 hours (once-daily regimens) of the intended time, otherwise to wait to take the next dose on time. In the event of vomiting after a dose, they should wait for the next dose before taking the study medication again in order avoid accidental overdose.

The doses of aspirin (aspirin lysine) and rivaroxaban will not be adjusted for any parameter. The maximum duration that a participant will receive aspirin (aspirin lysine) will be 42 days and rivaroxaban 28 days.

8.7 Dosage modifications

Discontinuation of aspirin (aspirin lysine) and/or rivaroxaban may be considered in the following circumstances:

Major bleeding, including life-threatening bleeding

Intolerable adverse reaction such as minor bleeding that cannot be controlled by local measures

Discovery of severe thrombocytopenia (platelet count < 50,000/μL)

No dose adjustment beyond that specified by the randomisation schedule will be permitted.

8.8 Known drug reactions and interaction with other therapies

Details are to be found in section 8.9 below, in the current SmPCs for aspirin (aspirin lysine) and rivaroxaban.

8.9 Concomitant medication

Recording of any prescribed or over-the-counter medication after randomisation will be made at each visit. If at visits 3 and 4, any of the criteria in section 7.10 are met, the participant will be withdrawn if the visit cannot be postponed. All individual medications, prescription and over-the-counter, will be recorded peri-event for any SAE or discontinuation due to AE. The following should be observed if any participant receives concomitant medication after enrolment:

Oral antiplatelet/anticoagulant therapies

Aspirin (acetylsalicylic acid): Aspirin use, with the exception of the study medication as prescribed, is prohibited during the study period. At enrolment, patients will be asked to not use aspirin as an analgesic and they will be made aware of the range of over-the-counter products that contain it. If no contraindication exists, paracetamol will be recommended if the need for analgesia arises. Patients will be asked about extra aspirin use, including over the counter supplies, at all visits. If, during the course of study treatment, a participant develops a clinical indication for combination antithrombotic therapy, a clinically appropriate regimen will be prescribed and they will be followed up but withdrawn from the study. Participants who receive at least one dose of IMP will be followed up by telephone 14(-2) days after withdrawal.

Other oral thrombotic therapies: Treatment with any other oral antithrombotic therapy apart from the study medication (e.g. clopidogrel, prasugrel, ticagrelor, dipyridamole, cilostazol, warfarin, dabigatran, edoxaban, apixaban) is prohibited during the course of study drugs. However, if during the course of study treatment, a participant develops a contraindication to aspirin or rivaroxaban, these will be discontinued/withheld and they will be withdrawn from the study. Participants who receive at least one dose of IMP will be followed up by telephone 14(-2) days after withdrawal.

As all participants will be taking rivaroxaban at some stage during the study, drugs that interact with its metabolism should be avoided. Strong inhibitors of CYP3A4 substantially increase plasma rivaroxaban levels whereas strong inducers of CYP3A4 have the opposite effects. Consequently, strong CYP3A4 inhibitors (eg, ketoconazole, itraconazole, voriconazole, telithromycin, clarithromycin [but not erythromycin or azithromycin], nefazadone, ritonavir, saquinavir, nelfinavir, indinavir, atanazavir, cobicistat or over 1 litre daily of grapefruit juice) should not be co-administered with rivaroxaban as plasma drug levels would be substantially increased. If regular treatment with such therapies is essential, then rivaroxaban should be withheld and they should be withdrawn from the study. Participants who receive at least one dose of IMP will be followed up by telephone 14(-2) days after withdrawal.

Co-administration of rivaroxaban with strong inducers of CYP3A should also be avoided (e.g. rifampin/rifampicin, rifabutin, phenytoin, carbamazepine, phenobarbital).

Similarly, co-administration of rivaroxaban with SSRIs or SNRIs has been associated with an elevation of bleeding risk so therapy with these agents should be avoided.

If regular treatment with such therapies becomes essential during the study medication period then they will be withdrawn from the study. Participants who receive at least one dose of IMP will be followed up by telephone 14(-2) days after withdrawal.

Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs may affect the antiplatelet and immunomodulatory effects of aspirin whilst increasing the risk of gastric irritation/ulceration and renal impairment. Levels of arachidonic acid metabolites may also be affected. Requirement for regular treatment with an NSAID at enrolment meets the exclusion criteria of the study. Treatment with NSAIDs during the study period is discouraged. COX2 inhibitors are prohibited in combination with study medication. Paracetamol is safe in combination with both aspirin and rivaroxaban

and therefore will be the recommended analgesic/antipyretic agent if required. In the case of a participant requiring treatment with an NSAID/paracetamol/COX2 inhibitor within 48 hours prior to visit 3, 4 or 5, they will be withdrawn if the visit cannot be postponed. Participants who receive at least one dose of IMP will be followed up by telephone 14(-2) days after withdrawal.

Diuretics

Diuretics, including loop (eg. furosemide, bumetanide), thiazide (eg. bendroflumethiazide, indapamide) and potassium-sparing agents (eg. spironolactone, eplerenone, amiloride), exert effects on renal prostaglandin synthesis and therefore may interfere with urinary prostaglandins. A participant who receives a diuretic within 48 hours prior to visit 3, 4 or 5 will be withdrawn if the visit cannot be postponed. Those who receive at least one dose of IMP will be followed up by telephone 14(-2) days after withdrawal.

Angiotensin converting enzyme (ACE) inhibitors and Angiotensin receptor blockers (ARBs)

ACE inhibitors (eg. ramipril, enalapril, lisinopril) and ARBs (eg. candesartan, losartan, irbesartan) can modestly affect renal prostaglandin production and hence urinary assays, however, a significant proportion of the patients in the target population group being studied in this trial are expected to be receiving these agents at enrolment. Therefore, receipt of an ACE inhibitor or ARB will not preclude randomisation or ongoing participation in the study. The crossover design and within-patient comparisons are anticipated to minimise any interfering effect.

Parenteral anticoagulants

In the event of an indication for parenteral anticoagulation developing, the participant will be withdrawn from the study but those who receive at least one dose of IMP will be followed up by telephone 14(-2) days after withdrawal.

Glycoprotein IIb/IIIa antagonists

In the event of an indication for an agent in this group developing, the participant will be withdrawn from the study. Participants who receive at least one dose of IMP will be followed up by telephone 14(-2) days after withdrawal.

Fibrinolytic agents

Treatment with fibrinolytic agents should be avoided whenever possible during treatment with study medication. If a participant is to receive a fibrinolytic agent the participant will be withdrawn from the study. Participants who receive at least one dose of IMP will be followed up by telephone 14(-2) days after withdrawal.

Methotrexate

Treatment with doses of methotrexate > 15 mg/week in combination with aspirin may increase the toxicity of methotrexate.

Uricosurics

Aspirin may reduce the effect of uricosurics (e.g. benzbromarone, probenecid) and so are not recommended in combination. Allopurinol may be given with low-dose aspirin.

Tenofovir

Administration of tenofovir disproxil fumarate with NSAIDs, including aspirin, can lead to a greater risk of renal failure so combining the two is not recommended.

Other medications to be used with caution

In addition to those medications contraindicated or not recommended above, caution is advised in use of the following drugs in combination with aspirin (aspirin lysine), as detailed in the current SmPC. Given participants will already receiving aspirin at enrolment, and total daily dose in this study will not exceed their baseline of 75 mg once-daily, there are no study-specific restrictions on prescription of these, but usual clinical considerations should be made.

• Glucocorticoids (with the exception of hydrocortisone replacement therapy) with analgesic and antipyretic doses of doses: increased risk of haemorrhage.

• Pemetrexed in patients with mild-to-moderate renal failure (creatinine clearance between 45 ml/min and 80 ml/min): increased risk of pemetrexed toxicity (following a reduction in the renal clearance of pemetrexed by aspirin) in the case of concomitant administration of anti-inflammatory doses of yylic acid.

• Antidiabetics (insulins, chlorpropamide): increase in the hypoglycemic effect of high doses of aspirin (hypoglycemic effect and sulfamic reinstatement of its plasma protein bond).

• Interferon alpha: risk of inhibition of the effect of interferon.

• Intrauterine device: (controversial) risk of reduction in the efficacy of the device.

• Diuretics, angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor antagonists with anti-inflammatory, analgesic or antipyretic doses of aspirin: acute renal failure may occur in dehydrated patients owing to a reduction in glomerular filtration secondary to a fall in renal prostaglandin production. In addition, a reduction in the antihypertensive effect may occur. Ensure that the patient is correctly hydrated and monitor renal function at the start of treatment.

• Methotrexate in doses < 15 mg/week: In the case of a drug combination of methotrexate and acetylsalicylic acid, the haematological toxicity of methotrexate may rise owing to aspirin inducing a reduction in renal clearance of methotrexate. During the first weeks of concomitant administration, blood counts should be checked every week. Closer supervision is necessary in patients with even mild renal function impairment and in elderly patients.

• Gastrointestinal topicals, antacids and charcoal: increase in renal excretion of acetylsalicylic acid following urine alkalinisation. It is recommended to administer gastrointestinal topicals and antacids at least 2 hours before or after aspirin.

• Digoxin and lithium: aspirin disrupts renal excretion of digoxin and lithium, bringing about an increase in their plasma concentration. It is recommended to monitor the plasma concentration of digoxin and lithium at the beginning and end of aspirin treatment. Dose adjustment may be necessary.

• Corticosteroids via the systemic route: the risk of gastrointestinal ulcers and bleeding may be increased in the case of concomitant administration of aspirin and corticosteroids.

• Ibuprofen: experimental data suggest that ibuprofen may inhibit the effect of low doses of aspirin on platelet aggregation in the case of concomitant administration. Nevertheless, the limitations of these data and uncertainties about the extrapolation of the data ex vivo to the clinical situation mean that it is

impossible to draw definite conclusions about the regular use of ibubrofen. A clinically significant effect is considered unlikely where ibuprofen is used occasionally.

• Valproic acid: Concomitant administration of salicylates and valproic acid may lead to decreased protein binding and inhibition of the metabolism of valproic acid causing increased free and total serum levels of valproic acid.

• Vaccine against chickenpox: it is recommended not to give salicylates for 6 weeks after administration of chickenpox vaccine. Cases of Reye's syndrome have occurred following the administration of salicylates during chickenpox infections.

• Phenytoin: the concomitant use of salicylates and phenytoin may lead to an increase in serum concentrations of phenytoin.

• Metamizole may reduce the effects of aspirin on platelet aggregation in the case of simultaneous administration. This combination must therefore be used with caution in patients taking low-dose aspirin for cardiovascular prophylaxis.

• Acetazolamide: caution is required when salicylates are administered concomitantly with acetazolamide owing to an increased risk of metabolic acidosis.

• Alcohol may increase risk of gastrointestinal injury if consumed concomitantly with aspirin. Caution should therefore be exercised in the event of alcohol consumption by patients taking aspirin.

• Nicorandil: In patients treated concurrently with nicorandil and NSAIDs, including aspirin lysine and other aspirin, the risk of serious complications such as gastrointestinal ulcers, gastrointestinal perforation and haemorrhage is increased.

• Levothyroxine: Salicylates, in particular at repeated or chronic doses higher than 2 g / d, can inhibit the binding of thyroid hormones to transporter proteins and therefore cause an initial transient increase in free thyroid hormones, followed by an overall decrease in thyroid hormone levels. Thyroid hormone levels should be checked. The dose of levothyroxine should be adapted if necessary.

8.10 Trial restrictions

There will be no specific restrictions on diet, exercise or lifestyle during the trial.

Surgery and other invasive procedures

If surgery or another procedure becomes necessary during the study medication period, study medication should be stopped and the participant will be withdrawn from the study. Participants who receive at least one dose of IMP will be followed up by telephone 14(-2) days after withdrawal.

The effects of aspirin, but not rivaroxaban, may be reversed by platelet transfusion, which might be considered in the event of life-threatening bleeding.

Similarly the effects of rivaroxaban, but not aspirin, may be reversed by prothrombin complex concentrate or, if available, andexanet alfa, which might be considered in the event of life-threatening bleeding.

8.11 Assessment of compliance with treatment

Compliance will be assessed at the end of each treatment period by counting the remaining drug sachets (aspirin lysine) and tablets (rivaroxaban) returned by the participant, and by direct observation of the participant taking last dose of aspirin (aspirin lysine) +/- rivaroxaban in each period. Sachet/tablet counts

will be carried out by the investigators and then cross-checked by the Pharmacy Department. Compliance will be recorded in the CRF.

8.12 Name and description of each Non-Investigational Medicinal Product (NIMP)

There are no NIMPs included in this study.

9 PHARMACOVIGILANCE

9.1 Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions. It is important to note that this is entirely separate to the known side effects listed in the SmPC. It is specifically a temporal relationship between taking the drug, the half-life, and the time of the event or any valid alternative aetiology that would explain the event.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect Other ‘important medical events’ may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which 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.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected	A serious adverse reaction, the nature and severity of which is not

Serious Adverse Reaction (SUSAR)	consistent with the information about the medicinal product in question set out in the reference safety information: • SmPC for aspirin (aspirin lysine) • SmPC for rivaroxaban

9.2 Operational definitions for (S)AEs

Adverse events and reactions will be recorded and reported from enrolment (visit 1) to visit 6 (14[-2] days after last dose of IMP).

All SAEs will be reported to the sponsor within 24 hours of the research team becoming aware of them. This will be using the sponsor's proforma which will be sent by email to a dedicated address provided specifically for this purpose. A copy will be kept in the trial master file and the AE log will be completed. All SAEs will be reviewed at the regular trial management group meetings.

All other AEs not meeting the criteria for reporting as serious will be recorded in the CRF.

The investigators will assess the relatedness of adverse events to the IMPs (aspirin [aspirin lysine], rivaroxaban).

9.3 Recording and reporting of SAEs, SARs AND SUSARs

SAEs/SUSARs will be recorded and reported from randomisation to visit 6 14(-2) days after last dose of IMP

These must be recorded on the Sheffield Teaching Hospitals NHS Foundation Trust SAE reporting Form and emailed to the Sponsor's dedicated email address for this purpose **within 24 hours** of the research staff becoming aware of the event.

For each **SAE or SUSAR** the following information will be collected:

- full details in medical terms and case description
- event duration (start and end dates, if applicable)
- action taken
- outcome
- seriousness criteria
- causality (i.e. relatedness to trial drug / investigation), in the opinion of the investigator
- whether the event would be considered anticipated.

Any change of condition or other follow-up information should be emailed to the Sponsor as soon as it is available or at least within 24 hours of the information becoming available. Events will be followed up until the event has resolved or a final outcome has been reached.

All SAEs assigned by the PI or delegate as both suspected to be related to IMP-treatment and unexpected will be classified as SUSARs and will be subject to expedited reporting to the Medicines and Healthcare Products Regulatory Agency (MHRA). The sponsor will inform the MHRA, the REC and the relevant Marketing Authorisation Holder(s) of SUSARs within the required expedited reporting timescales.

9.4 Responsibilities

Principal Investigator (PI) or delegate:

1. Checking for AEs and ARs when participants attend for treatment / follow-up.
2. Using medical judgement in assigning seriousness, causality and whether the event/reaction was anticipated using the Reference Safety Information approved for the trial.
3. Ensuring that all SAEs are recorded and reported to the sponsor within 24 hours of becoming aware of the event and provide further follow-up information as soon as available. Ensuring that SAEs are chased with Sponsor if a record of receipt is not received within 2 working days of initial reporting.
4. Ensuring that AEs and ARs are recorded and reported to the sponsor in line with the requirements of the protocol.

Chief Investigator (CI, in this single-site study the same individual as the PI) or delegate:

1. Clinical oversight of the safety of patients participating in the trial, including an ongoing review of the risk / benefit.
2. Using medical judgement in assigning whether and event/reaction was anticipated or expected in line with the Reference Safety Information.
3. Immediate review of all SUSARs.
4. Assigning Medical Dictionary for Regulatory Activities (MedDRA) or Body System coding to all SAEs and SARs.
5. Preparing the clinical sections and final sign off of the Development Safety Update Report (DSUR).
6. Collection and verification of AEs, ARs, SAEs, SARs and SUSARs according to the trial protocol.

Sponsor: (NB where relevant these can be delegated to CI)

1. Expedited reporting of SUSARs to the Competent Authority (MHRA) and REC within required timelines.
2. Checking for (annually) updates to the Reference Safety Information for the trial.
3. Preparing standard tables and other relevant information for the DSUR in collaboration with the CI and ensuring timely submission to the MHRA and REC.

9.5 Notification of deaths

- Any deaths will be treated as an SAE and reported accordingly to the sponsor using the SAE reporting procedures within 24 hours of becoming aware, irrespective of whether the death is related to the IMP or an unrelated event.

9.6 Pregnancy reporting

- Pregnancy is not expected to occur as women of childbearing potential who are pregnant or unwilling to use reliable contraception will be excluded from study involvement. Nevertheless, any pregnancy occurring in a study participant should be reported to the Chief Investigator and the Sponsor using the relevant sponsor-provided Pregnancy Reporting Form within 24 hours of notification. Any pregnant participant will be withdrawn from the study. Follow-up of the pregnant participant and child born to a pregnant trial participant, if this becomes necessary, will be discussed with the sponsor in this unlikely event.

- There are no restrictions nor requirements for follow up of the children of male participants in the study.

9.7 Overdose

An overdose will be defined as any amount taken, above that prescribed, that in the opinion of a medically-qualified investigator has the potential to cause significant harm. If an overdose of a study drug occurs, then investigators or other site personnel will inform the Sponsor immediately that they become aware of it, and in any case within 24 hours. Overdoses may be observed from sachet/tablet counts or patient comment.

In case of overdose of aspirin (aspirin lysine), this will be considered equivalent to standard preparations of aspirin. Aspirin is an established agent and standard guidelines for the assessment and management of overdose should be followed.

Similarly, rivaroxaban is an established drug and usual clinical procedures will be followed in the event of overdose.

Participants who receive an overdose of medication may be withdrawn from the study at the discretion of the investigators.

If an SAE is associated with the overdose, the investigators will ensure the overdose is fully described in the SAE report form.

9.8 Reporting urgent safety measures

If any urgent safety measures are taken, the CI/Sponsor shall immediately and, in any event, no later than 3 days from the date the measures are taken, give written notice to the MHRA and the relevant REC of the measures taken and the circumstances giving rise to those measures.

9.9 The type and duration of the follow-up of participants after adverse reactions.

Adverse drug reactions will be followed up by telephone or in person, as necessary, until resolved or stable.

Follow up of existing SUSARs (i.e. those which start within the reporting period) will need to be reported to the sponsor indefinitely until resolved.

9.10 Development safety update reports

The CI will provide (in addition to the expedited reporting above) DSURs once a year throughout the clinical trial, or as necessary, to the Competent Authority (MHRA), where relevant the Research Ethics Committee and the sponsor.

The report will be submitted within 60 days of the Developmental International Birth Date (DIBD) of the trial each year until the trial is declared ended.

10 STATISTICS AND DATA ANALYSIS

10.1 Sample size calculation

The primary hypothesis of this study is that the difference in post-dose bleeding time between aspirin (aspirin lysine) 75 mg once-daily and aspirin (aspirin lysine) 20 mg twice-daily plus rivaroxaban 2.5 mg twice-daily will be less than the difference in bleeding time between aspirin (aspirin lysine) 75 mg once-daily and aspirin (aspirin lysine) 75 mg once-daily plus rivaroxaban 2.5 mg twice-daily.

Statistical power can be estimated based on previous data from our group showing a mean difference in peak effect bleeding time of 154.5 seconds (18%) between aspirin (aspirin lysine) 20 mg BD (plus ticagrelor 90 mg twice-daily, another antithrombotic drug) and 75 mg OD (also plus ticagrelor 90 mg twice-daily), with an inter-individual standard deviation of 316 seconds.

Assuming a similar proportionate difference in bleeding time between the aspirin (aspirin lysine) 20 mg BD- and aspirin (aspirin lysine) 75 mg OD-based combination regimens seen in our previous study, and a worst-case scenario where the intra-individual standard deviation of the difference is 316 secs (likely to be less because of effective pairing of bleeding time data seen in our previous data), we would have 80% power to detect a difference in the change in bleeding time from aspirin (aspirin lysine) 75 mg OD to aspirin (aspirin lysine) 20 mg BD + rivaroxaban 2.5 mg BD vs. aspirin (aspirin lysine) 75 mg OD to aspirin (aspirin lysine) 75 mg OD plus rivaroxaban 2.5 mg BD, of 115 seconds (one-tailed paired-t test), and 90% power to detect a difference of 135 secs.

Based on our previous data for aspirin (aspirin lysine) 75 mg OD + ticagrelor as a baseline, proportionally this would represent a difference of 14% (power 0.8) or 16% (power 0.9), which we would class as meaningful differences with the potential to reduce the incidence or severity of clinical bleeding events.

Sample size calculations have not been prepared for the secondary endpoints as these are exploratory.

10.2 Planned recruitment rate

It is aimed to recruit participants at the minimum rate of one per week. Based on our group's previous experience of similar studies, this is sensibly feasible.

10.3 Statistical analysis plan

10.3.1 Summary of baseline data and flow of patients

The following baseline data will be collected and reported:

From visit 1

- Demographic data (age, sex, ethnicity)
- Vital signs: pulse, blood pressure and temperature
- Weight and BMI
- Full blood count, urea & electrolytes, liver function tests

Categorical data will be reported as proportions and percentages. Continuous data will be reported as mean and standard deviation if normally distributed otherwise median and interquartile range. Logarithmic transformation will be considered if data are skewed.

Differences between the dosing regimens and timepoints groups will be assessed by paired t-tests and by repeated measures ANOVA with timepoint and treatment as factors.

A CONSORT flow diagram will be prepared for inclusion in the report of study findings.

10.3.2 Primary outcome analysis

The primary endpoint will be based on bleeding time at 2 hours following the last dose of IMP of each treatment period and will be determined as the difference in bleeding time between aspirin (aspirin lysine) 75 mg once-daily alone and either aspirin (aspirin lysine) regimen in combination with rivaroxaban 2.5mg bd, allowing comparison of the increase in bleeding time with aspirin (aspirin lysine) 20 mg twice-daily plus rivaroxaban 2.5 mg twice-daily vs. aspirin (aspirin lysine) 75 mg once-daily plus rivaroxaban 2.5 mg twice-daily, compared with a one-tailed paired t-test.

10.3.3 Secondary outcome analysis

Secondary analyses will be as follows:

1. Bleeding time immediately before the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.
2. Fibrin clot lag time immediately before the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.
3. Fibrin clot lag time 2 hours following the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.
4. Fibrin clot lysis time immediately before the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.
5. Fibrin clot lysis time 2 hours following the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

6. Plasma TNFα immediately before the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.
7. Plasma TNFα 2 hours following the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.
8. Plasma IL-6 immediately before the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.
9. Plasma IL-6 2 hours following the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.
10. Serum CRP at 2 hours following the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.
11. Circulating leukocyte count immediately before the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.
12. Circulating leukocyte count 2 hours following the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.
13. Serum TXB_2 immediately before the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.
14. Serum TXB_2 2 hours following the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.
15. Urinary PGI-M immediately before the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD

vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

16. Urinary PGI-M 2 hours following the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.
17. Urinary TxM immediately before the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.
18. Urinary TxM 2 hours following the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.
19. Maximum platelet aggregation responses to arachidonic acid (0.3 and 1 mmol/L), collagen (4 and 16 μg/mL) and ADP (20 μmol/L) immediately before the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using a repeated measures ANOVA, with pairwise comparisons if found to be significant.
20. Maximum platelet aggregation responses to arachidonic acid (0.3 and 1 mmol/L), collagen (4 and 16 μg/mL) and ADP (20 μmol/L) 2 hours after the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using a repeated measures ANOVA, with pairwise comparisons if found to be significant.
21. Final clot turbidity immediately before the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.
22. Final clot turbidity 2 hours following the last dose of IMP of the treatment period assessed within-patients between treatment regimens (aspirin [aspirin lysine] 20 mg BD plus rivaroxaban 2.5 mg BD vs aspirin [aspirin lysine] 75 mg OD vs aspirin [aspirin lysine] 75 mg OD plus rivaroxaban 2.5 mg BD) using repeated measures ANOVA, with pairwise comparisons if found to be significant.

10.4 Subgroup analyses

No pre-specified subgroup analyses are planned.

10.5 Adjusted analysis

If variables are found to be of skewed distribution, logarithmic transformation will be performed.

10.6 Interim analysis and criteria for the premature termination of the trial

No interim analyses are planned.

10.7 Participant population

- The pharmacodynamic analysis set will include all participants who achieve at least 80% compliance with study medication during each of the three periods.
- The safety analysis set (for the purposes of adverse event reporting etc.) will include any participant randomised into the trial that received at least one dose of IMP.

10.8 Procedure(s) to account for missing or spurious data

- Missing data will be recorded by notating 'NR' (not recorded) in the relevant section of the CRF
- Where analysis is performed using ANOVA, missing data will be estimated by multiple imputation using the IBM SPSS software package. Sensitivity analyses will be carried out to report the robustness of this approach.

10.9 Other statistical considerations.

Not applicable to this study

10.10 Economic evaluation

Not applicable to this study.

11 DATA MANAGEMENT

11.1 Data collection tools and source document identification

Source data will be recorded on the study-specific paper case report form (CRF). A separate paper CRF will be used to record laboratory data. Source data will feed from the CRF to the trial master database.

A paper trial master file will be kept within the Cardiovascular Research Unit at the University of Sheffield, maintained by the Research Co-ordinator.

11.2 Data handling and record keeping

The investigators will maintain a study database, including an audit trail of data changes ensuring that there is no deletion of entered data, and will utilise a security system to protect against unauthorized access. They will2 maintain a list of the individuals authorized to make data changes, maintain adequate backup of the data, and archiving of any source data (i.e. hard copy and electronic). This will include a clear

record of data changes if transformed during processing. The investigators will use an unambiguous participant identification code that allows identification of all the data reported for each participant. All electronic data will be kept securely stored on University of Sheffield systems, or external handlers contracted by the University of Sheffield for this purpose. The Sponsor will be responsible for ensuring compliance with the requirements outlined above.

11.3 Access to Data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections in line with participant consent.

11.4 Archiving

Archiving will be authorised by the Sponsor following submission of the end of trial report. The sponsor will archive all study documents through existing SOPs and external contracts for a minimum of 15 years after the end of the study, as per local protocols. Destruction of essential documents will require authorisation from the Sponsor. The trial database will be kept by the investigators for an undefined period of time, but at least 15 years, in electronic format on University of Sheffield file storage systems.

12 MONITORING, AUDIT & INSPECTION

- A Trial Monitoring Plan will be developed and agreed by the Trial Management Group (TMG) and CI based on the trial risk assessment, which may include on-site monitoring. This will be dependent on a documented risk assessment of the trial by the Sponsor.
- It is anticipated that monitoring audits will take place after the first participant first visit and last participant last visit.
- The monitoring plan will be kept in the trial master file.
- The monitoring personnel will be determined by the Sponsor.
- The processes reviewed will include participant enrolment, consent, eligibility, and allocation to trial groups; adherence to trial interventions and policies to protect participants, including reporting of harm and completeness and timeliness of data collection.
- The research team, headed by the PI/CI, will be responsible for verification of the accuracy of study data.
- Monitoring will be performed through site visit to review original documentation.
- The investigators will host the site visits.

13 ETHICAL AND REGULATORY CONSIDERATIONS

13.1 Research Ethics Committee (REC) review and report, and Health Research Authority (HRA) approval

- Before the start of the trial, approval will be sought from a REC for the trial protocol, informed consent forms and other relevant documents e.g. advertisements and GP information letters. HRA approval will also be obtained.

- Substantial amendments that require review by REC will not be implemented until the REC grants a favourable opinion for the amendment and HRA amendment approval is received. Amendments may also need to be reviewed and accepted by the MHRA and/or NHS R&D departments before they can be implemented in practice.
- All correspondence with the REC, MHRA and HRA will be retained in the Trial Master File.
- An annual progress report (APR) will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended.
- It is the Chief Investigator's responsibility to produce the annual reports as required.
- The Chief Investigator will notify the REC of the end of the trial.
- if the trial is ended prematurely, the Chief Investigator will notify the REC, including the reasons for the premature termination.
- Within one year after the end of the trial, the Chief Investigator will submit a final report with the results, including any publications/abstracts, to the REC.

13.2 Peer review

The study design has been reviewed and approved by multiple independent scientific/medical personnel on behalf of the MRC Confidence in Concepts funding scheme, who also reviewed the statistical aspects.

13.3 Public and Patient Involvement

The concepts tested in this study have been previously presented to the Sheffield Cardiovascular Patient Panel.

13.4 Regulatory Compliance

- The trial will not commence until a Clinical Trial Authorisation (CTA) is obtained from the MHRA and Favourable REC opinion.
- The protocol and trial conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004 and any relevant amendments.
- No ionising radiation will be used during this study.

13.5 Protocol compliance

- Prospective, planned deviations or waivers to the protocol are not allowed under the UK regulations on Clinical Trials and must not be used e.g. it is not acceptable to enrol a participant if they do not meet the eligibility criteria or restrictions specified in the trial protocol.
- Accidental protocol deviations must be adequately documented on the relevant Sponsor-provided forms and reported to the Chief Investigator and Sponsor immediately.
- Deviations from the protocol which are found to frequently recur will require immediate action and could potentially be classified as a serious breach, at the discretion of the Sponsor.

13.6 Notification of Serious Breaches to GCP and/or the protocol

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

The Sponsor will be notified immediately of any case where the above definition applies during the trial conduct phase

- The Sponsor will notify the licensing authority in writing of any serious breach of
  (a) the conditions and principles of GCP in connection with that trial; or
  (b) the protocol relating to that trial, as amended from time to time, within 7 days of becoming aware of that breach

13.7 Data protection and patient confidentiality

All investigators and trial site staff must comply with the requirements of the Data Protection Act 2018 with regards to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles.

- Personal information will be collected by the investigators, kept secure within a room in the Cardiovascular Research Unit that is kept locked and alarmed out-of-hours, and will be maintained by the staff of the Cardiovascular Research Unit. This will involve:
  - Within the master trial database the creation of coded, depersonalised data whereby the participant's identifying information will be replaced by the study enrolment number.
  - Secure maintenance of the data and the linking code in separate locations using encrypted digital files within password protected folders and storage media on NHS or University of Sheffield computing systems.
  - Limiting access to the minimum number of individuals necessary for quality control, audit, and analysis.
- Monitoring visits will take place at site to avoid any data breaches. Transmission of information relating to safety eg. SAE reports will occur by secure NHS email channels. Any sharing of data with collaborators will be anonymised.
- Study source documents will be kept for a minimum of 15 years as per Sponsor protocols.
- The data custodian will be the CI.

13.8 Financial and other competing interests for the chief investigator, PIs at each site and committee members for the overall trial management

Robert F. Storey: institutional research grants/support from AstraZeneca; consultancy fees from AstraZeneca, Bayer, Bristol Myers Squibb/Pfizer alliance, Haemonetics, Idorsia, Novartis and Thromboserin; speaker fees from AstraZeneca, and Bayer and Bristol Myers Squibb/Pfizer alliance. Nominated by University of Sheffield as inventor on patent application related to concepts explored in this study.

William A. E. Parker: nominated by University of Sheffield as inventor on patent application related to concepts explored in this study.

13.9 Indemnity

The NHS indemnity scheme will apply for harm arising from management of research and research conduct. Additionally, The University of Sheffield will provide insurance against liabilities for which it may be legally liable and this cover will include any such liabilities arising out of this research project/study.

13.10 Amendments

Any changes to the protocol or informed consent form will be assessed by the Sponsor to determine the necessary approvals to be obtained (MHRA, HRA and/or REC). The Sponsor will then approve the amendment when the necessary approvals have been granted.

13.11 Post-trial care

Whilst not able to offer study-specific medical care once a participant's involvement in the study ends, the investigators will ensure that participants are signposted to the relevant NHS services should these be needed.

13.12 Access to the final trial dataset

- The investigators identified on the delegation log will have access to the full dataset, at the discretion of the CI.

14 DISSEMINATION POLICY

14.1 Dissemination policy

- The data arising from the trial will be owned by University of Sheffield.
- On completion of the trial, the data will be analysed and tabulated and a Final Trial Report prepared.
- The trial report will be accessible via the EudraCT system, in a medical journal and on request from the CI.
- The CI will retain the sole right to publish any of the trial data.
- The MRC and the Sheffield NIHR Clinical Research Facility will be acknowledged within any publications but will not have review/publication rights of the data from the trial.
- Participants will be able to obtain a short summary of the results by letter on request.
- It will not be possible for the participant to specifically request results from the investigators.
- The disclosure of the trial protocol, full trial report, anonymised participant level dataset, and statistical code for generating the results may be made available to interested parties at the discretion of the CI, not before 1 year after study completion.

14.2 Authorship eligibility guidelines and any intended use of professional writers

Those individuals who contribute significantly to the development, conduct and writing-up of the study will be offered authorship of the final trial report. Individually-named authors will meet the authorship criteria of The International Committee of Medical Journal Editors. No professional medical writers will be involved in the preparation of reports or publications.

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