The University Of Sheffield.

Sheffield Teaching Hospitals NHS Foundation Trust

The impact of aspirin dose modification, with or without ticagrelor, on the innate immune response:

WILL lOWer dose aspirin Therapy ReducE the response to Endotoxin? – (WILLOW TREE)

Clinical Study Protocol

RESEARCH REFERENCE NUMBERS

STH20370

TRIAL REGISTRY NUMBER

Clinicaltrials.gov: TBC

OTHER RESEARCH REFERENCE NUMBERS

EudraCT: (2018-004285-35)
MHRA: 21304/0268/001-0001
IRAS ID: 254420

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: 20/09/2021

Name (please print):

Dr Dipak Patel

Position:

Research & Innovation Manager

Chief Investigator:

Signature: RF Storey

Date: 19/09/2021

Professor Robert Storey

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
Co-Investigator	Dr William Parker Clinical Research Fellow in Cardiology Cardiovascular Research Unit Northern General Hospital Barnsley Road Drive, Sheffield S5 7AU Telephone 0114 2266159 Fax 0114 3052008 Email w.parker@sheffield.ac.uk
Trial Co-ordinator	Dr Hannah McMellon Senior Research Co-Ordinator Cardiovascular Research Unit Centre for Biomedical Research Northern General Hospital Barnsley Road Drive, Sheffield S5 7AU Telephone 0114 2266159 Fax 0114 3052008 Email h.mcmellon@sheffield.ac.uk
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	British Heart Foundation: BHF Clinical Research Training Fellowship no. FS/18/49/33752 - William Parker “The impact of aspirin dose modification on the innate immune response”
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	Angiotensinogen 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
CI	Chief Investigator
COX	Cyclo-oxygenase
CRF	Case Report Form
CRP	C-reactive Protein
CTA	Clinical Trial Authorisation
CYP	Cytochrome P450
DAPT	Dual Antiplatelet Therapy
DSUR	Development safety update report
EP	Prostanglandin 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
IL-1	Interleukin 1
IL-6	Interleukin 6
IMP	Investigation 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 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 of Healthcare Research
NIMP	Non-investigational medicinal product
NR	Not Recorded
NSAID	Non-Steroidal Anti-Inflammatory Drug
OD	Once-daily
$P2Y_{12}$	Platelet adenosine diphosphate receptor
PCI	Percutaneous Coronary Intervention
PGE_2	Prostaglandin E2
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 A2
µg	Micrograms
UK	United Kingdom
µL	Microlitres
µmol	Micromoles
USA	United States of America

iii. TRIAL SUMMARY

Trial Title	The impact of aspirin dose modification, with or without ticagrelor, on the innate immune response: WILL lOWer dose aspirin Therapy ReducE the response to Endotoxin? – (WILLOW TREE)	
Internal ref. no.	STH20370	
Clinical Phase	Phase IV	
Trial Design	Randomised controlled hybrid parallel-group and crossover study	
Trial Participants	Healthy volunteers aged 18-65 years, male or women of non-childbearing potential	
Planned Sample Size	72	
Treatment duration	2 x 10 (to 14) day periods	
Follow up duration	Until 10 days after last IMP/endotoxin dose	
Planned Trial Period	January 2019 –February 2022	
	Objectives	**Outcome Measures**
Primary	Assess inflammatory response to intravenous endotoxin	Plasma tumour necrosis factor α
Secondary	Assess inflammatory response to intravenous endotoxin	Plasma interleukin 6 Serum C-reactive protein Circulating leukocyte count (and subsets)
	Assess effects on key arachidonic acid metabolites during endotoxaemia	Serum thromboxane B_2 Serum prostaglandin E_2 Urine prostacyclin metabolite
	Assess effects on platelet function and haemostasis during endotoxaemia	Bleeding time Platelet aggregation responses to arachidonic acid, collagen and adenosine diphosphate
Investigational Medicinal Product(s)	1. Aspirin (aspirin lysine) 2. Ticagrelor Additionally, sterile bacterial endotoxin will be administered to model inflammation (regarded as a NIMP for the purpose of this protocol)	
Formulation, Dose, Route of Administration	1. 100 mg sachets for dissolution, administered orally as 20 mg twice-daily, 75 mg once-daily or 300 mg once-daily 2. 90 mg oro-dispersible tablets administered as a single loading dose of 180 mg	

iv. FUNDING AND SUPPORT IN KIND

FUNDERS	FINANCIAL AND NON FINANCIAL SUPPORT GIVEN
British Heart Foundation (BHF)	Funding of the study: BHF Clinical Research Training Fellowship no. FS/18/49/33752 - William Parker: "The impact of aspirin dose modification on the innate immune response"
Northern General Hospital Clinical Research Facility (NGH-CRF)	Provision of space and services within the NGH-CRF in kind to conduct the study

v. ROLE OF TRIAL SPONSOR AND FUNDER

The sponsor, Sheffield Teaching Hospitals NHS Foundation Trust, will contribute to study design and arrangements before approving the protocol and associated study documents. The sponsor will handle site initiation, monitoring and close-out. They will also provide pharmacy and clinical support to the study.

The funder, British Heart Foundation, 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 this is an exploratory study utilising healthy volunteers rather than patients, and will not change usual clinical care, 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), as well as Professor Ian Sabroe, Professor Bianca Rocca and Dr Ramzi Ajjan.

Dr Heather Judge assisted with designing laboratory methods.

Dr Erica Wallis, Dr Nana Theodorou and Mrs Sam Walmsley critically reviewed the protocol on behalf of the sponsor.

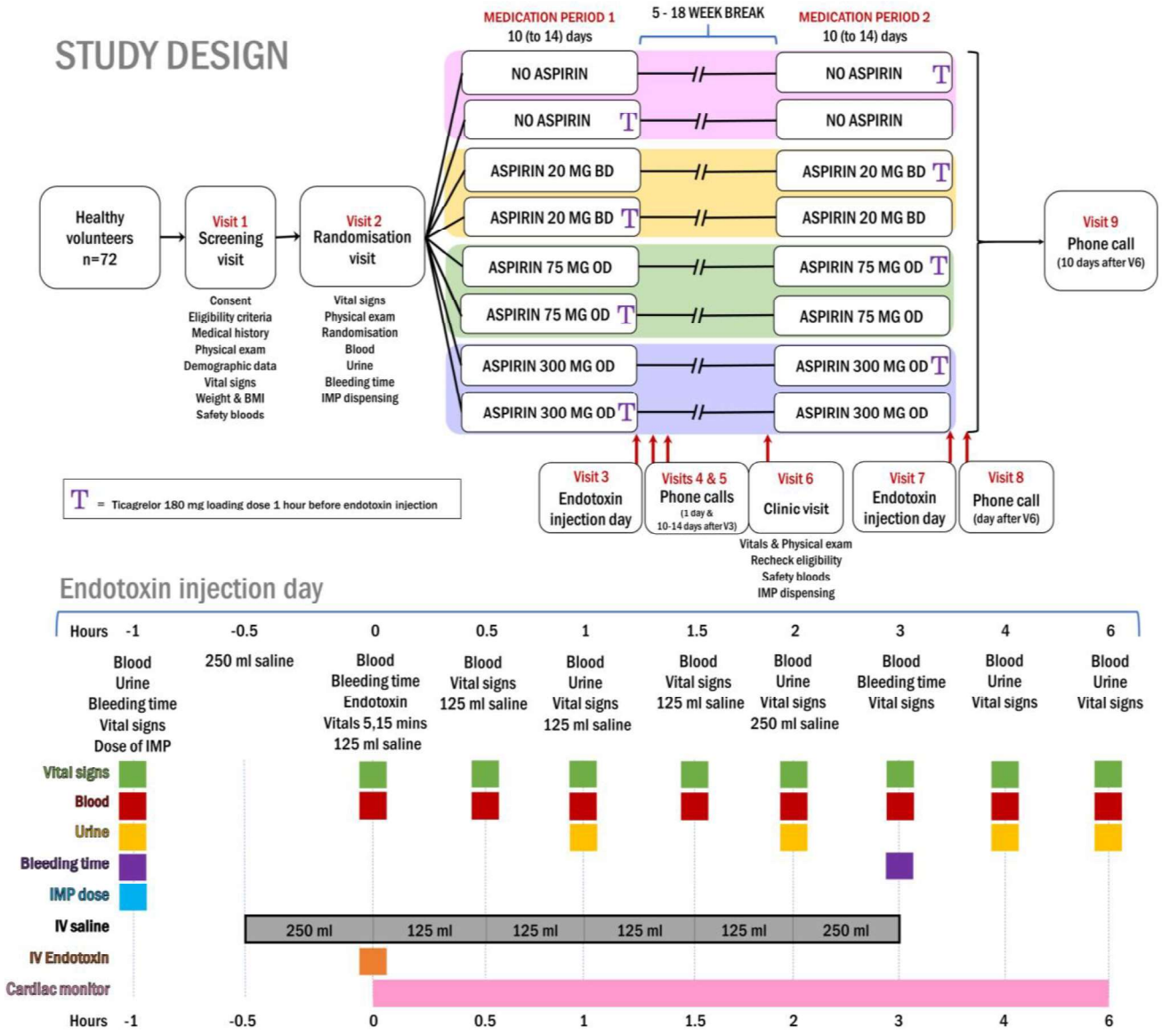
Mrs Kim Ryalls provided input into the pharmacy arrangements detailed in the protocol.

Representatives of the British Heart Foundation reviewed the protocol from a scientific and statistical point-of-view.

At an early stage in development, the study design and patient information sheet were reviewed by the Sheffield Cardiovascular Patient Panel and suggestions for changes incorporated into further revisions.

viii. KEY WORDS: Aspirin, ticagrelor, inflammation, arachidonic acid metabolites, platelets, haemostasis

ix. TRIAL FLOW CHART



1 BACKGROUND

Acute coronary syndromes (ACS) are responsible for over 33,000 deaths a year in the UK alone. Atherothrombosis is the central pathological process in the development of ACS, driven by inflammation which leads to formation of lipid-rich plaques that may then rupture or erode, leading to activation of platelets and coagulation factors, vessel occlusion and distal ischaemia [1]. Levels of cytokines, such as tumour necrosis factor α (TNF-α) and interleukin 6 (IL-6) , strongly correlate with outcomes in ACS [2].

The PLATelet inhibition and patient Outcomes (PLATO) study showed that dual antiplatelet therapy (DAPT) with the irreversible cyclo-oxygenase (COX) inhibitor aspirin (acetylsalicylic acid) and the platelet $P2Y_{12}$ receptor antagonist ticagrelor was superior to the previous standard regimen of aspirin and clopidogrel in ACS, reducing the incidence of major adverse cardiovascular events (MACE). Consequently, the combination of aspirin 75 mg OD and ticagrelor 90 mg BD now represents a first-line recommended regimen of DAPT in ACS [3, 4]. Despite such potent antiplatelet therapy, the MACE risk remains around 10% in 1 year [5].

Recently, reducing inflammation has been identified as a rewarding strategy for tackling residual risk and further improving outcomes after ACS. Significantly, chronic administration of a monoclonal antibody against a pro-inflammatory cytokine, interleukin-1 (IL-1), reduced the incidence of MACE in patients with a history of myocardial infarction (MI) and evidence of ongoing systemic inflammatory response [6]. However, this approach was associated with an increased risk of fatal infection and so other strategies for reducing systemic inflammation are a priority for future research.

We found that platelet $P2Y_{12}$ inhibitors attenuate the inflammatory response in an established *in vivo* endotoxaemia model [7] and have used this technique again to study the effect of hypoglycaemia on inflammatory response [8].

Aspirin is a non-selective, irreversible inhibitor of cyclooxygenase (COX) 1 and 2 enzymes, at lower doses exhibiting relative pharmacokinetic selectivity for platelet COX1 [9], 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 entirely counteracting this [10].

Aspirin has established benefits of preventing recurrent thrombotic events after acute MI [11] but, despite this, trials of long-term aspirin therapy carried out in high-risk patients with conditions associated with chronic inflammation, such as diabetes mellitus or peripheral vascular disease, have been unable to show convincing beneficial effects on the frequency of MACE [12-16].

2 RATIONALE

In addition to its antiplatelet effects, aspirin has immunomodulatory effects. On the one hand, including at a dose of 75 mg OD, it may have anti-inflammatory effects mediated via generation of 15-epi-lipoxin A_4 [17], whilst, on the other hand, there is growing evidence that aspirin may increase certain factors key to the progression of atherothrombosis. As well as observed effects *in vitro* and *ex vivo*, it has now been

shown that aspirin, tested at a dose of 80 mg OD, potentiates both *in vivo* TNF-α and IL-6 release in response to endotoxaemia and that, although a standard regimen of ticagrelor appears to counteract these effects to an extent, significant augmentation of inflammatory response by aspirin persists despite this [18].

The mechanism for this effect has not been fully elucidated, but may be related to inhibition of platelet-derived prostaglandin E_2 (PGE_2), another eicosanoid synthesised by platelets and leukocytes in response to endotoxin [18, 19] that can inhibit the synthesis and release of pro-inflammatory cytokines from monocytes via its action on prostaglandin receptors EP2 and EP4 [20, 21]. Endotoxin binds to toll-like receptor 4 (TLR4) on the surface of monocytes, resulting in signalling including upregulation of nuclear factor kappa B (NFκB), with an increase in synthesis and release of pro-inflammatory cytokines, including TNF-α and IL-6, mediating vascular inflammation [22, 23]. The action of PGE_2 on EP2 can also reduce the translation and surface expression of TLR4 [24]. In addition to binding endotoxin, TLR4 also functions as a damage receptor, activated by a range of factors released during atherogenesis, endothelial injury and thrombosis, including fibrin degradation products and damage-associated molecular patterns [25, 26]. An established link also exists between bacterial infection, for example pneumonia, which results in inflammation via the endotoxin-TLR4 pathway, and elevated risk of MACE [27]. This makes endotoxaemia a highly relevant model of inflammation to use in the study of atheroinflammation, in contrast to other models that may not be so pathophysiologically pertinent to cardiovascular disease and may not be potentiated by aspirin [28, 29]. Aspirin, a COX inhibitor, may reduce release of PGE_2, therefore potentiating the pro-inflammatory response to endotoxin. COX inhibition may also hypothetically result in increases in other non-COX derived eicosanoids such as leukotriene B_4 (LTB_4), which antagonises the effect of PGE_2 via action on the leukotriene BLT1 receptor [30].

It has been proposed that aspirin could safely be stopped in ticagrelor-treated patients with a history of percutaneous coronary intervention (PCI), however this approach was not superior in a recent large randomised clinical trial [31], although another large study is ongoing [32]. Although ticagrelor monotherapy may indeed be sufficient to prevent stent thrombosis, there are likely to remain significant numbers of patients at high-risk of ongoing native plaque rupture events in whom DAPT is needed to optimise protection against future MACE, since aspirin and ticagrelor have additive antithrombotic effects [33, 34]. Aspirin, in the setting of DAPT, may therefore provide advantages of augmenting the antithrombotic effect of $P2Y_{12}$ inhibition but may hypothetically result in disadvantageous effects upon progression of atherothrombosis. If this profile could be optimised, then outcomes may be improved. It is also plausible that imbalance in these factors might contribute to the lack of benefit of ticagrelor over clopidogrel in patients treated with higher doses of aspirin in the PLATO study [35], although this remains to be fully explored.

We completed a pilot study of patients with a history of ACS receiving aspirin and ticagrelor, and have shown that a novel regimen of very-low-dose twice-daily aspirin (Aspirin lysine) (20 mg BD), when compared to the standard regimen of 75 mg OD, may lead to lower circulating levels of IL-6 and leukocyte count. We also observed that, compared to standard dosing, the novel regimen had reduced peak antithrombotic effect but maintained a similar trough effect, and this was associated with an improvement in haemostasis when assessed by bleeding time, which is a useful measure for assessing the impact of antithrombotic

drug combinations (in contrast to limited sensitivity for effects of antithrombotic monotherapy). There was also a trend towards increased levels of prostacyclin metabolite, suggesting reduced COX2 inhibition [36].

2.1 Assessment and management of risk

Aspirin and ticagrelor are both widely used as antiplatelet drugs for the treatment of cardiovascular disease. Combining aspirin and ticagrelor increases the risk of bleeding over that of either treatment when given alone, but it is standard clinical practice (and within ticagrelor's licence) to do so, and we will limit participant's exposure to a single loading dose of ticagrelor. Interactions with other drugs will not be an issue as those receiving any regular medication at screening will be excluded from participation.

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).

Ticagrelor

Reported side effects of ticagrelor include the following (reproduced from the current SmPC [37]):

Category	Very common	Common	Uncommon
Neoplasms benign, malignant and unspecified (including cysts and polyps)			Tumour bleeding[a]
Blood and lymphatic system disorders	Blood disorder bleeding[b]		
Immune system disorders			Hypersensitivity including angioedema[c]
Metabolism and nutrition disorders	Hyperuricaemia[d]	Gout/Gouty Arthritis	
Psychiatric disorders			Confusion
Nervous system disorders		Dizziness, syncope, headache	Intracranial haemorrhage
Eye disorders			Eye haemorrhage[e]
Ear and labyrinth disorders		Vertigo	Ear haemorrhage
Vascular disorders		Hypotension	
Respiratory, thoracic and mediastinal disorders	Dyspnoea	Respiratory system bleeding[f]	
Gastrointestinal disorders		Gastrointestinal haemorrhage[g], diarrhoea, nausea, dyspepsia, constipation	Retroperitoneal haemorrhage
Skin and subcutaneous tissue disorders		Subcutaneous or dermal bleeding[h], rash, pruritus	
Musculoskeletal connective tissue and bone			Muscular bleeding[i]
Renal and urinary disorders		Urinary tract bleeding[j]	
Reproductive system and breast disorders			Reproductive system bleeding[k]
Investigations		Blood creatinine increased[d]	
Injury, poisoning and procedural complications		Post procedural haemorrhage, traumatic bleeding[l]	

a e.g. bleeding from bladder cancer, gastric cancer, colon cancer

b e.g. increased tendency to bruise, spontaneous haematoma, haemorrhagic diathesis

c Identified in post-marketing experience

d Frequencies derived from lab observations (Uric acid increases to >upper limit of normal from baseline below or within reference range. Creatinine increases of >50% from baseline.) and not crude adverse event report frequency.

e e.g. conjunctival, retinal, intraocular bleeding

f e.g. epistaxis, haemoptysis

g e.g. gingival bleeding, rectal haemorrhage, gastric ulcer haemorrhage

h e.g. ecchymosis, skin haemorrhage, petechiae

i e.g. haemarthrosis, muscle haemorrhage

j e.g. haematuria, cystitis haemorrhagic

k e.g. vaginal haemorrhage, haematospermia, postmenopausal haemorrhage

l e.g. contusion, traumatic haematoma, traumatic haemorrhage

Endotoxin

Escherichia coli endotoxin at a dose of 2 ng/kg induces a controlled, safe inflammatory response peaking at 2 hours and subsiding by 6 hours [38]. This can typically be associated with symptoms of fever, headache, myalgia/arthralgia and occasionally rigors. On average, body temperature rises to 38^{o}C. Rare cases of bradycardia and hypotension have been reported. These symptoms and signs typically peak at 2-3 hours after administration then return to normal by 6 hours.

Over the past 20 years, over a thousand healthy volunteers have taken part in similar studies of endotoxaemia and it is exceptionally rare for it to have any serious effects.

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 are in good general health
- excluding those with significant co-morbidity or receiving any concomitant medication
- as short as feasible exposure to study drugs (eg. using a single dose of ticagrelor rather than a more prolonged period of dual antiplatelet therapy) whilst maintaining scientific credibility
- intravenous hydration before and after endotoxin administration, and nursing of participants supine during challenges
- performing endotoxin challenges in the Clinical Research Facility within a large acute hospital (including 24 hour emergency medical and critical care cover)
- supervision of endotoxin challenges by a study physician
- frequent measurement of vital signs during endotoxin challenges

- continuous cardiac monitoring during endotoxin challenges
- rigorous safety monitoring by the study team and sponsor
- a track record of safely performing similar studies including antiplatelet medication and endotoxin administration to healthy volunteers by our group and others

3 OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

3.1 Primary objective

The primary objective of this study is to assess, in healthy volunteers, the effects of 3 regimens of aspirin (Aspirin lysine), or no aspirin, on the release of TNF-α during endotoxaemia.

It is hypothesised that TNF-α will be significantly lower when receiving aspirin 20 mg BD compared to 75 mg OD and 300 mg OD.

3.2 Secondary objectives

The secondary objectives of this study are to assess, during endotoxaemia, the effects of 3 regimens of aspirin (Aspirin lysine) or no aspirin, with and without a loading dose of ticagrelor, on:

1. Plasma TNF-α (comparisons other than those stated in the primary objective)
2. Plasma IL-6
3. Serum CRP
4. Leukocyte count (and subsets)
5. Serum TXB_2
6. Serum PGE_2
7. Urine PGI-M
8. Bleeding time
9. Platelet aggregation responses to arachidonic acid (AA), collagen and adenosine diphosphate (ADP).

3.3 Outcome measures/endpoints

Primary endpoint/outcome

The primary endpoint will be plasma TNF-α at 2 hours post-injection of 2 ng/kg endotoxin compared between participants receiving no IMP, aspirin (Aspirin lysine) 20 mg BD, aspirin (Aspirin lysine) 75 mg OD and aspirin (Aspirin lysine) 300 mg OD.

Secondary endpoints/outcomes

1. Plasma levels from 0 to 6 hours after endotoxin administration, of TNF-α
2. Plasma levels from 0 to 6 hours after endotoxin administration, of plasma TNF-α
3. Change in serum CRP from 0 to 6 hours after endotoxin administration
4. Leukocyte count, from 0 to 6 hours after endotoxin administration
5. Serum TXB_2 from 0 to 6 hours after endotoxin administration

6. Serum PGE_2, from 0 to 6 hours after endotoxin administration
7. AUC of the graph, from 0 to 6 hours after endotoxin administration, of urine PGI-M
8. AUC of the graph, from 0 to 3 hours after endotoxin administration, of bleeding time
9. Mean platelet aggregation responses to arachidonic acid (AA), collagen and adenosine diphosphate (ADP), from 0 to 6 hours after endotoxin administration.

Secondary endpoints will be compared between:

1. Participants receiving no IMP, aspirin (aspirin lysine) 20 mg BD, aspirin (aspirin lysine) 75 mg OD and aspirin (aspirin lysine) 300 mg OD.
2. Participants receiving no aspirin and a 180 mg loading dose of ticagrelor, aspirin (aspirin lysine) 20 mg BD plus a 180 mg loading dose of ticagrelor, aspirin (aspirin lysine) 75 mg OD plus a 180 mg loading dose of ticagrelor, and aspirin (aspirin lysine) 300 mg OD plus a 180 mg loading dose of ticagrelor.
3. Participants receiving no IMP and a 180 mg loading dose of ticagrelor.
4. Participants receiving aspirin (aspirin lysine) 20 mg BD, and aspirin (aspirin lysine) 20 mg BD plus a 180 mg loading dose of ticagrelor.
5. Participants receiving aspirin (aspirin lysine) 75 mg OD, and aspirin (aspirin lysine) 75 mg OD plus a 180 mg loading dose of ticagrelor.
6. Participants receiving aspirin (aspirin lysine) 300 mg OD, and aspirin (aspirin lysine) 300 mg OD plus a 180 mg loading dose of ticagrelor.
7. Participants receiving no IMP, aspirin (aspirin lysine) 20 mg BD, aspirin (aspirin lysine) 75 mg OD and aspirin (aspirin lysine) 300 mg OD, no aspirin and a 180 mg loading dose of ticagrelor, aspirin (aspirin lysine) 20 mg BD plus a 180 mg loading dose of ticagrelor, aspirin (aspirin lysine) 75 mg OD plus a 180 mg loading dose of ticagrelor, and aspirin (aspirin lysine) 300 mg OD plus a 180 mg loading dose of ticagrelor.

Analyses 2-7 will also be performed for plasma TNF-α (as secondary endpoints). For further details see section 10 of this protocol.

4 TRIAL DESIGN

The trial is a pharmacodynamic study to determine the effect of aspirin dose modification on immune response, compared to control and compared between the presence and absence of potent $P2Y_{12}$ inhibition with ticagrelor. Healthy volunteers will receive either no aspirin (control group) or one of three doses of aspirin (aspirin lysine) with or without a loading dose of ticagrelor on the last day of the first medication period, as specified in this protocol, for 10-14 days. They will then receive E. coli endotoxin to induce an immune response. Serial measurements of inflammatory markers, cytokines, leucocyte function, prostanoids and platelet function will be taken over 6 hours. Bleeding time will be measured before endotoxin administration and 3 hours after it to assess haemostasis. After a washout period of at least 5 weeks, and not more than 18 weeks, subjects will then crossover to receive the same regimen of aspirin (aspirin lysine) (or no aspirin in the control group) without or with a loading dose of ticagrelor on the last day of the second medication period. The dose-dependent effects of aspirin and the impact of ticagrelor will be compared between the groups.

A total of 72 eligible subjects are planned to be randomised. There will be three regimens of aspirin (aspirin lysine) included in the study, in addition to a control group (no aspirin), each tested with and without a 180 mg loading dose of ticagrelor. Aspirin (aspirin lysine) 75 mg OD represents the current standard regimen in ACS and for primary and secondary prevention of cardiovascular disease. Aspirin (aspirin lysine) 20 mg BD

is a novel regimen hypothesised to provide a better profile of inflammatory and antithrombotic effect than 75 mg OD. Lastly, aspirin 300 mg OD represents a regimen commonly given after coronary artery bypass grafting.

Randomisation will be in a 1:1:1:1:1:1:1:1 fashion and managed by a commercially available service for this purpose, SealedEnvelope, with whom the sponsor will have a study-specific contract.

E. coli endotoxin will be administered at the end of each treatment period according to the protocol. Subjects will be recruited from the area local to the Northern General Hospital, Sheffield. For example, we will recruit members of staff and students from universities in Sheffield and from Sheffield Teaching Hospitals. Volunteers will be given as much time as they need to consider taking part in the study prior to signing informed consent.

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 healthy volunteers, recruited primarily from local higher education establishments, but also staff from local healthcare institutions.

6 PARTICIPANT ELIGIBILITY CRITERIA

6.1 Inclusion criteria

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

1. Healthy male subjects, or female subjects not of childbearing potential (either surgically sterile or post-menopausal)
2. Age between 18 and 65 years inclusive
3. Non-smokers
4. Body mass index (BMI) between 18 and 28 kg/m^2 inclusive, with a body weight between 60-100 kg
5. In good health as determined by a medical history, physical examination, vital signs and clinical laboratory test results, including renal and liver function, and full blood count
6. Provision of informed consent before any trial-related activity

6.2 Exclusion criteria

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

1. Any history of cancer, diabetes or, in the opinion of the investigator, clinically-significant cardiovascular, respiratory, metabolic, renal, hepatic, gastrointestinal, haematological, dermatological, neurological, psychiatric or other major disorders.
2. Any history of either significant multiple drug allergies or known allergy to the study drugs or any medicine chemically related to the study drugs.

3. A clinically-significant illness within the preceding 2 weeks.
4. Any clinically-significant abnormal laboratory test result (full blood count, urea & electrolytes [sodium, potassium, urea and creatinine], liver function tests, clotting screen, urinalysis), at screening (visit 1), in the opinion of the investigator.
5. A supine blood pressure at screening, after resting for 5 minutes, higher than 150/90 mmHg or lower than 105/65 mmHg.
6. A supine heart rate at screening, after resting for 5 minutes, outside the range of 50-100 beats/min.
7. Receipt of any prescribed or over-the-counter systemic or topical medication within the preceding 48 hours.
8. Planned or expected requirement, during the next 3 months (at randomisation, or 3 weeks at the start of period 2), for any non-study systemic or topical prescribed drug, or for systemic or topical over-the-counter NSAID, corticosteroid, antihistamine or any other drug that could affect inflammation, thrombosis or haemostasis in the opinion of the investigator.
9. Receipt of an investigational medicinal product, excluding those for the purposes of this study, within the previous four month period (new chemical entity) or three month period (licensed product) or subjects who have received a vaccine within the previous three weeks.
10. Any donation of blood or plasma in the preceding one month period, excluding for the purposes of this study.
11. A history of alcohol or drug abuse.
12. Mental incapacity or language barriers that preclude adequate understanding.
13. Any other factor that, in the opinion of the investigator, would affect the participant's ability to safely and reliably complete the study, or would affect the scientific validity of the results obtained.

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 dose-dependent effects of aspirin on the immune response to endotoxaemia including on inflammatory markers, cytokines, prostanoids, leucocytes, haemostasis and platelet function, and (ii) show whether the addition of ticagrelor modifies these effects.

We will measure inflammatory markers and cytokines that include, but are not limited to, TNFα, IL-6 and CRP.

To determine the detailed effects of aspirin dose modification on circulating prostanoids in the unstimulated and endotoxin-stimulated states, we will measure a panel of eicosanoids and/or their metabolites, including, but not limited to, PGE_2, PGI_2 and TXB_2 and will additionally assess the effect of ticagrelor on endotoxin-induced responses.

Haemostasis will be assessed by measuring bleeding time pre- and 3 hours post-endotoxin using a method shown to be sensitive to additive effects of antiplatelet agents [33]. The procedure for measuring bleeding time is described in the study-specific SOP relating to this.

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, post-medication at peak inflammation and during the resolution phase of inflammation, 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 (eg. 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

Potential participants will self-identify through established channels of advertisement to local Universities and NHS staff and students, using documents approved for this purpose by the relevant research authorities.

7.1.1 Participant identification

Potential participants will initially self-identify, based on our advertisement, and medically-qualified members of the research team will then determine eligibility.

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) measured supine after 5 minutes' rest.
- Weight and BMI
- Recording of any concomitant medication
- Safety blood tests: 12.5 ml blood sample for full blood count, urea and electrolytes (sodium, potassium, urea, creatinine), liver function tests and clotting screen

- Urinalysis

7.1.3 Payment

Volunteers who receive at least one endotoxin injection will receive £100 per endotoxin injection (maximum £200) to reimburse them for their time, inconvenience and any discomfort caused. Transportation to and from the Clinical Research Facility will be provided if necessary.

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 eight treatment sequences, in a 1:1:1:1:1:1:1:1 fashion:

- No IMP for 10-14 days, then ≥ 5 week washout period, then no aspirin for 10-14 days, but a loading dose of ticagrelor (180 mg) given one hour before endotoxin injection (sequence 1).
- No aspirin for 10-14 days, but a loading dose of ticagrelor (180 mg) given one hour before endotoxin injection, then ≥ 5 week washout period, then no IMP for 10-14 days (sequence 2).
- Aspirin (aspirin lysine) 20 mg BD for 10-14 days, then ≥ 5 week washout period, then aspirin (aspirin lysine) 20 mg BD for 10-14 days plus a loading dose of ticagrelor (180 mg) given one hour before endotoxin injection (sequence 3).

- Aspirin (aspirin lysine) 20 mg BD for 10-14 days plus a loading dose of ticagrelor (180 mg) given one hour before endotoxin injection, then ≥ 5 week washout period, then aspirin (aspirin lysine) 20 mg BD for 10-14 days (sequence 4).
- Aspirin (aspirin lysine) 75 mg OD for 10-14 days, then ≥ 5 week washout period, then aspirin (aspirin lysine) 75 mg OD for 10-14 days plus a loading dose of ticagrelor (180 mg) given one hour before endotoxin injection.
- Aspirin (aspirin lysine) 75 mg OD for 10-14 days plus a loading dose of ticagrelor (180 mg) given one hour before endotoxin injection, then ≥ 5 week washout period, then aspirin (aspirin lysine) 75 mg OD for 10-14 days (sequence 6).
- Aspirin (aspirin lysine) 300 mg OD for 10-14 days, then ≥ 5 week washout period, then aspirin (aspirin lysine) 300 mg OD for 10-14 days plus a loading dose of ticagrelor (180 mg) given one hour before endotoxin injection (sequence 7).
- Aspirin (aspirin lysine) 300 mg OD for 10-14 days plus a loading dose of ticagrelor (180 mg) given one hour before endotoxin injection, then ≥ 5 week washout period, then aspirin (aspirin lysine) 300 mg OD for 10-14 days (sequence 8).

7.3.1 Method of implementing the randomisation/allocation sequence

Randomisation will be handled by an online interactive web-based randomisation service, sealedenvelope.com, using a simple randomisation method.

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, if appropriate, a study-specific prescription for study medication for period 1. A separate prescription will be issued for period 2 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) measured supine after 5 minutes' rest
- Weight and BMI
- Concomitant medication
- Lab safety parameters: full blood count, serum urea & electrolytes (sodium, potassium, urea and creatinine), liver function tests, clotting screen; and urinalysis

At visit 2

- Vital signs: pulse, blood pressure and temperature
- Physical examination
- TNF-α, IL-6, serum TXB_2, urine PGI-M, platelet aggregation responses to arachidonic acid, ADP and collagen
- Bleeding time

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: 12.5 ml blood sample for full blood count, urea & electrolytes (sodium, potassium, urea and creatinine), liver function tests, clotting screen; and urinalysis

If eligible, subjects will be asked to return to the Clinical Research Facility, Northern General Hospital, on Day 1 for the experimental part of the study. They will be randomised to receive a two-period medication regimen (Figure 3), with each period consisting of 10 to 14 days of aspirin (aspirin lysine) or no aspirin prior to the experimental days, with ticagrelor loading dose at the end of one period, 1 hour prior to endotoxin administration, and without ticagrelor loading dose at the end of the other.

There will be a washout period of at least 5 weeks between the first experimental day and the second medication period. A letter will be sent to the participant's GP to inform them of enrolment in the study. Volunteers who complete the study will receive £100 per medication period/experimental day to reimburse them for their time, inconvenience and any discomfort caused. 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)

- Vital signs
- Physical examination
- 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
- 27 ml venous blood samples for baseline cytokines, prostanoids, inflammatory markers, platelet function, and plasma, serum, DNA and RNA for storage
- Bleeding time measurement
- Urine sample (around 20 ml will be taken for study purposes) for baseline prostanoid measurement
- Provided with supply of study antiplatelet medication for period 1 as determined by randomisation
- Dosing training for use of aspirin lysine if required

Period 1: Day 1 - Day 10 (to 14)

- Participants will receive one of the following antiplatelet medication regimens for 10 to 14 days:
    - No IMP
    - No aspirin but a loading dose of ticagrelor (180 mg) taken on the last day of the medication period, 1 hour prior to endotoxin injection
    - Aspirin (aspirin lysine) 20 mg BD (only)
    - Aspirin (aspirin lysine) 20 mg BD, and a loading dose of ticagrelor (180 mg) taken on the last day of the medication period, 1 hour prior to endotoxin injection
    - Aspirin (aspirin lysine) 75 mg OD (only)

- Aspirin (aspirin lysine) 75 mg OD, and a loading dose of ticagrelor (180 mg) taken on the last day of the medication period, 1 hour prior to endotoxin injection
- Aspirin (aspirin lysine) 300 mg OD (only)
- Aspirin (aspirin lysine) 300 mg OD, and a loading dose of ticagrelor (180 mg) taken on the last day of the medication period, 1 hour prior to endotoxin injection

Visit 3 - Period 1: Day 11 (to 15)

On arrival

- Vital signs
- Physical examination
- Adverse event recording
- Concomitant medication recorded
- IMP compliance check for period 1
- Medically-qualified member of the research team to confirm that the withdrawal criteria (section 7.10) are not met, prior to proceeding further with visit

-1 hour (1 hour before endotoxin injection)

- IV cannula insertion (x2, one in each arm)
- 35.5 ml venous blood sample for baseline lab safety (full blood count, urea & electrolytes [sodium, potassium, urea and creatinine], liver function tests, clotting screen), cytokines, prostanoids, inflammatory markers and platelet function
- Bleeding time measurement
- Urine sample for prostanoid assessment at baseline
- Receive last dose of aspirin of period 1, or last dose of aspirin plus a loading dose of ticagrelor (180 mg), or no IMP, as directed by randomisation allocation
- Collect unused study medication and perform drug accountability check (sachet/pill count)
- Infusion of 250 ml 0.9% saline over 30 minutes
- 23 ml venous blood at 60 minutes after last dose of oral antiplatelet medication or equivalent time if not receiving IMP during period 1 (just before endotoxin administration)

0 hour (time of endotoxin injection)

- Continuous cardiac monitoring starts
- *E.coli* endotoxin 2 ng/kg intravenously administered over 1 minute
- Vital signs at 5, 15 and 30 minutes post-endotoxin
- Continuous IV infusion of 250 ml 0.9% saline over 1 hour
- 14 ml venous blood sample at 30 minutes post endotoxin

1 hour post endotoxin injection

- Vital signs at 1 hour and 1.5 hours post endotoxin injection
- Continuous IV infusion of 250 ml 0.9% saline over 1 hour
- 14 ml venous blood sample at 1 hour post endotoxin
- Urine sample at 1 hour post endotoxin
- 14 ml venous blood sample at 1½ hours post endotoxin

2 hours post-endotoxin injection

- Vital signs at 2 hours post endotoxin
- Continuous IV infusion of 250 ml 0.9% saline over 1 hour
- 23 ml venous blood sample at 2 hours post endotoxin
- Urine sample at 2 hours post endotoxin

3 hours post-endotoxin injection

- Vital signs at 3 hours post endotoxin
- 23 ml venous blood sample at 3 hours post endotoxin
- Bleeding time measurement at 3 hours post endotoxin

4 hours post-endotoxin injection

- Vital signs at 4 hours post endotoxin
- 14 ml venous blood sample at 4 hours post endotoxin
- Urine sample at 4 hours post endotoxin

6 hours post-endotoxin injection

- Vital signs at 6 hours post endotoxin
- 14 ml venous blood sample at 6 hours post endotoxin
- Urine sample at 6 hours post endotoxin
- Continuous cardiac monitoring ends

Visit 4 (Period 1, 1 day after visit 3)

- Telephone follow-up for adverse events
- Facility to convert to in-person visit if any concerns raised

There will then follow a washout period of at least 5 weeks, and not more than 18 weeks, between the first endotoxin challenge day and the beginning of the second medication period. This is to avoid reliably any tolerance of the response to endotoxin, a phenomenon absent by 5 weeks [39].

Visit 5 (10 to 14 days after visit 3)

- Telephone follow-up for adverse events

Visit 6 (Period 2, Day 0)

- Vital signs (BP, pulse, temperature)
- Physical examination
- Adverse event recording
- Concomitant medication recording
- Medically qualified investigator to confirm eligibility criteria again (see section 6) and that no withdrawal criteria (section 7.10) are met at this point prior to proceeding to medication period 2
- Laboratory safety blood tests: 12.5 ml venous blood for full blood count, urea & electrolytes (sodium, potassium, urea and creatinine), liver function tests, clotting screen
- Medication dispensed for period 2
- Dosing training for use of aspirin lysine if required

Participants will be advised to begin antiplatelet medication, allocated as follows, for period 2 from the morning after visit 6:

  - If received no IMP in period 1, to receive no aspirin, but a loading dose of ticagrelor (180 mg) 1 hour before endotoxin injection in period 2.

- If received no aspirin but a loading dose of ticagrelor (180 mg) 1 hour before endotoxin injection in period 1, to receive no IMP in period 2.
- If received aspirin (aspirin lysine) 20 mg BD (only) in period 1, to receive aspirin (aspirin lysine) 20 mg BD plus a loading dose of ticagrelor (180 mg) 1 hour before endotoxin injection in period 2.
- If received aspirin (aspirin lysine) 20 mg BD plus a loading dose of ticagrelor (180 mg) 1 hour before endotoxin injection in period 1, to receive aspirin (aspirin lysine) 20 mg BD (only) in period 2.
- If received aspirin (aspirin lysine) 75 mg OD (only) in period 1, to receive aspirin (aspirin lysine) 75 mg OD plus a loading dose of ticagrelor (180 mg) 1 hour before endotoxin injection in period 2.
- If received aspirin (aspirin lysine) 75 mg OD plus a loading dose of ticagrelor (180 mg) 1 hour before endotoxin injection in period 1, to receive aspirin (aspirin lysine) 75 mg OD (only) in period 2.
- If received aspirin (aspirin lysine) 300 mg OD (only) in period 1, to receive aspirin (aspirin lysine) 300 mg OD plus a loading dose of ticagrelor (180 mg) 1 hour before endotoxin injection in period 2.
- If received aspirin (aspirin lysine) 300 mg OD plus a loading dose of ticagrelor (180 mg) 1 hour before endotoxin injection in period 1, to receive aspirin (aspirin lysine) 300 mg OD (only) in period 2.

Period 2, Days 1 to 10 (to 14)

- Participants take antiplatelet medication for period 2 as determined by the randomisation allocation (figure 3)

Visit 7 (Period 2, Day 11 [to 15])

On arrival

- Vital signs
- Physical examination
- Adverse event recording
- Concomitant medication recorded
- IMP compliance check for period 2
- Medically-qualified member of the research team to confirm that the withdrawal criteria (section 7.10) are not met, prior to proceeding further with visit

-1 hour (1 hour before endotoxin injection)

- IV cannula insertion (x2, one in each arm)
- 35.5 ml venous blood sample for baseline lab safety (full blood count, urea & electrolytes [sodium, potassium, urea and creatinine], liver function tests, clotting screen), cytokines, prostanoids, inflammatory markers and platelet function
- Bleeding time measurement
- Urine sample for prostanoid assessment at baseline
- Receive last dose of aspirin of period 1, or last dose of aspirin plus a loading dose of ticagrelor (180 mg), or no IMP, as directed by randomisation allocation
- Collect unused study medication and perform drug accountability check (sachet/pill count)
- Infusion of 250 ml 0.9% saline over 30 minutes
- 23 ml venous blood at 60 minutes after last dose of oral antiplatelet medication or equivalent time if not receiving IMP during period 1 (just before endotoxin administration)

0 hour (time of endotoxin injection)

- Continuous cardiac monitoring starts
- *E.coli* endotoxin 2 ng/kg intravenously administered over 1 minute
- Vital signs at 5, 15 and 30 minutes post-endotoxin
- Continuous IV infusion of 250 ml 0.9% saline over 1 hour
- 14 ml venous blood sample at 30 minutes post endotoxin

1 hour post-endotoxin injection

- Vital signs at 1 hour and 1.5 hours post endotoxin injection
- Continuous IV infusion of 250 ml 0.9% saline over 1 hour
- 14 ml venous blood sample at 1 hour post endotoxin
- Urine sample at 1 hour post endotoxin
- 14 ml venous blood sample at 1½ hours post endotoxin

2 hours post-endotoxin injection

- Vital signs at 2 hours post endotoxin

- Continuous IV infusion of 250 ml 0.9% saline over 1 hour
- 23 ml venous blood sample at 2 hours post endotoxin
- Urine sample at 2 hours post endotoxin

3 hours post-endotoxin injection

- Vital signs at 3 hours post endotoxin
- 23 ml venous blood sample at 3 hours post endotoxin
- Bleeding time measurement at 3 hours post endotoxin

4 hours post-endotoxin injection

- Vital signs at 4 hours post endotoxin
- 14 ml venous blood sample at 4 hours post endotoxin
- Urine sample at 4 hours post endotoxin

6 hours post-endotoxin injection

- Vital signs at 6 hours post endotoxin
- 14 ml venous blood sample at 6 hours post endotoxin
- Urine sample at 6 hours post endotoxin
- Continuous cardiac monitoring ends

Visit 8 (Period 2, 1 day after visit 6)

- Telephone follow-up for adverse events
- Facility to convert to in-person visit if any concerns raised

Visit 9 (Period 2, 10 to 14 days after visit 6)

- Telephone follow-up for adverse events

Study investigations will be performed at the following time points:*

*Baseline refers to visit 2, and the pre- and post-endotoxin samples are to be taken during both experimental periods.

- **Inflammatory markers and cytokines**
- TNF-α: Baseline, pre-last medication dose, pre-endotoxin and 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin
- IL-6: Baseline, pre-last medication dose, pre-endotoxin and 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin
- CRP: Baseline, pre-endotoxin and 6 hours post-endotoxin

- **Prostanoids and Leukotrienes**
- Serum TXB_2: Baseline, pre-last medication dose, pre-endotoxin and 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin
- Urinary PGI-M: Baseline, pre-endotoxin and 1, 2, 4 and 6 hours post-endotoxin
- Urinary creatinine: Baseline, pre-endotoxin and 1, 2, 4 and 6 hours
- Serum PGE_2: Baseline, pre-endotoxin and 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin

- **Leucocyte phenotype and activation status**
- White blood cell count with differential leukocyte count: Baseline, pre-last medication dose, pre-endotoxin and 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin

- **Haemostasis**
- Bleeding time: Pre-endotoxin and 3 hours post-endotoxin

- **Platelet function tests**
- Light transmittance aggregometry (agonists: arachidonic acid 0.3 and 1 mmol/l, collagen 4 and 16 mg/ml, and ADP 20 µmol/l): Baseline, pre-endotoxin and 3 hours post-endotoxin

- **Samples for storage**
- Plasma for storage: Baseline, pre-last medication dose, pre-endotoxin and 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin
- Serum for storage: Baseline, pre-last medication dose, pre-endotoxin and 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin
- DNA/RNA for storage: Baseline
- Urine for storage: Baseline, pre-endotoxin and 1, 2, 4 and 6 hours post-endotoxin

Details of fluid management

We anticipate that the total fluid load per volunteer will be 1000 ml over the 8 hours of the day at the NGH-CRF (250 ml preload and 750 ml infusion). The volunteers will also receive fluid boluses if required, which would be typically be in the range of 250-500 ml normal saline, given if mean arterial blood pressure is reduced by 20% or more or to maintain a systolic blood pressure over 100 mmHg and/or to relieve symptoms related to hypotension. Under normal circumstances, we would give a maximum of 1000 ml of

0.9% saline over 1 hour and a maximum of 2000 ml of 0.9% saline over the 8 hours of the day at the NGH-CRF.

7.8 Long term follow-up assessments

Participants will be followed up 10 (to 14) days after the second endotoxin injection day (Period 2, Day 21) by telephone. Any new adverse events will be recorded. Participants will be thanked for their involvement in the study and arrangements made to provide the monetary reimbursement due to them. 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 the second endotoxin injection day. 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 insert intravenous cannula and/or 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 an 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)
- At the time of planned endotoxin injection, receipt of a non-study medication in the preceding 48 hours that, in the opinion of the investigator, is likely to affect inflammation, thrombosis or haemostasis, unless the endotoxin injection visit can be appropriately postponed (see section 7.10.1)
- Poor compliance (<80% of doses taken) with study aspirin (aspirin lysine) during the preceding medication period, unless the endotoxin visit can be appropriately postponed (see section 7.10.1) to achieve 80 % compliance
- 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 or endotoxin will be followed up by telephone at 10-14 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.

Withdrawn participants who have proceeded to randomisation will not be replaced.

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:

- an intercurrent illness, for example a viral upper respiratory tract infection;
- requirement for a course of medication that, in the opinion of the investigators, is likely to affect measures of inflammation, thrombosis or haemostasis; or
- compliance with aspirin (aspirin lysine) is <80% during the preceding study medication period

then if the subsequent endotoxin injection visit cannot be postponed to a time within the permitted window of 10-14 days into the medication period at which the illness or relevant effect of medication has deemed to have resolved or compliance has increased to at least 80% for the medication period, study medication may be stopped and the medication period restarted once the intercurrent illness or requirement for medication is deemed to have resolved, or when compliance is likely to be at least 80%. In this instance, an unscheduled visit will be arranged, the existing supply of aspirin (aspirin lysine) will be collected from the participant and returned to pharmacy for counting and destruction, and a new supply of aspirin (aspirin lysine) 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.

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] or 'Cardirene' [Sanofi-Aventis] '100 mg' sachets)
    - Each aspirin lysine '100 mg' sachet contains 180 mg aspirin lysine, equivalent to 100 mg acetylsalicyclic acid (aspirin). In this protocol the stated dose refers to the aspirin content.
    - Participants will be asked to prepare doses of 20 mg twice daily orally, where directed by the protocol, by dissolving 100 mg (1 sachet) in 100ml of drinking water and ingesting 20 ml of the solution using a graduated syringe, discarding the remainder.

- Participants will be asked to prepare doses of 75 mg once daily orally, 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.
- Participants will be asked to prepare doses of 300 mg once daily orally, where directed by the protocol, by dissolving 300 mg (3 sachets) in 100 ml of drinking water and ingesting the solution.
- A new sachet (or sachets) will be used for each dose of study medication

- Ticagrelor 90 mg oro-dispersible tablets (AstraZeneca, 10 tablets per pack)
  - Participants will be asked to take a loading dose of 180 mg (two orodispersible 90 mg tablets) 1 hour before endotoxin injection where specified by the randomisation allocation. This will be directly observed by the research team.

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.
- Ticagrelor 90 mg orodispersible tablets are licensed in the UK, in combination with aspirin, for the treatment of ACS.
- We will retain the original presentation of drugs as supplied without modification, but will be asking participants to vary the doses of aspirin (aspirin lysine) 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 ticagrelor 90 mg orodispersible 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.

Ticagrelor 90 mg orodispersible 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. Both aspirin (aspirin lysine) and ticagrelor will be segregated as clinical trial stock for the purposes of this study.

Aspirin (aspirin lysine) will be supplied to the investigators upon receipt of a suitable signed study-specific prescription at the start of each period. Only medically-qualified members of the study team will be able to complete the prescription. Where participants are required to take aspirin (aspirin lysine) by the randomisation allocation, one box of 30 sachets will be dispensed per period for each participant randomised to receive either aspirin 20 mg BD or aspirin 75 mg OD and two boxes of 30 sachets will be dispensed per period for each participant randomised to receive aspirin 300 mg OD.

Packs of ten 90 mg oro-dispersible ticagrelor tablets will be dispensed from the pharmacy to the investigators, on request, for storage in a dedicated area within the NGH-CRF. The pack will then be split and used to administer doses to a number of participants. Prescription, which will only be able to be made by a medically-qualified member of the study team, and administration will be recorded on a Sheffield Teaching Hospitals Drug Administration Record. A drug accountability log and temperature monitoring records will be kept for the ticagrelor stored in the NGH-CRF.

There are no specific post-dispensing storage instructions.

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

In the event of postponing visit 3 or 7 because of intercurrent illness and restarting a medication period from the beginning, unused medication from the period prior to the postponement will be returned to pharmacy and a new study-specific prescription will be made by a medically-qualified investigator for a new supply of aspirin (aspirin lysine).

8.5 Preparation and labelling of Investigational Medicinal Product

Labels for aspirin (aspirin lysine) and ticagrelor 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) as described elsewhere in this protocol.

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 endotoxin injection days 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 endotoxin injection days 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 ticagrelor will not be adjusted for any parameter. The maximum duration that a participant will receive aspirin (aspirin lysine) will be 28 days and ticagrelor 1 day.

8.7 Dosage modifications

Discontinuation of aspirin (aspirin lysine) and/or withholding ticagrelor 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 ticagrelor orodispersible tablets.

8.9 Concomitant medication

Regular concomitant medication will lead to exclusion from randomisation. Recording of any prescribed or over-the-counter medication after randomisation will be made at each subsequent visit. If at visits 3 and 7, any of the criteria in section 7.10 are met, the participant will be withdrawn if the visit cannot be postponed. Similarly, if at visit 6 the criteria in section 6 are not 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 randomisation:

Oral antiplatelet 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, but endotoxin injection should not be performed if paracetamol has been received in the preceding 24 hours. 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 regular antiplatelet 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 or endotoxin will be followed up by telephone 10-14 days after withdrawal.

Other oral antiplatelet therapies: Treatment with any other oral antiplatelet therapy apart from the study medication (e.g. clopidogrel, prasugrel, dipyridamole, cilostazol) is prohibited during the course of study medication. However, if during the course of study treatment, a participant develops a contraindication to

aspirin or ticagrelor, these will be discontinued/withheld and they will be withdrawn. Participants who receive at least one dose of IMP or endotoxin will be followed up by telephone 10-14 days after withdrawal.

As all participants will be taking ticagrelor at some stage during the study, drugs that interact with its metabolism should be avoided. Strong inhibitors of CYP3A4 substantially increase plasma ticagrelor 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, or over 1 litre daily of grapefruit juice) should not be co-administered with ticagrelor as plasma levels of ticagrelor would be substantially increased. If regular treatment with such therapies is essential, then ticagrelor should be withheld and they should be withdrawn. Participants who receive at least one dose of IMP or endotoxin will be followed up by telephone 10-14 days after withdrawal.

Concomitant therapy with simvastatin or lovastatin at doses higher than 40 mg daily is not permitted since ticagrelor significantly increases the levels of these statins and theoretically therefore may increase the risk of myopathy. There are no restrictions to other statin therapies (ie, doses of simvastatin or lovastatin ≤40 mg daily or any dose of any other statin is permitted) but if a participant fails to meet the eligibility criteria for the study (section 6) at the next check (visit 3, 6 or 7), they will be withdrawn if the visit cannot be postponed. Standard monitoring of patients for possible statin-associated myopathy should be carried out.

Co-administration of ticagrelor with CYP3A substrates with a narrow therapeutic index (e.g. cyclosporine and quinidine) should be avoided.

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

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 or endotoxin will be followed up by telephone 10-14 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 ticagrelor 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, if they fail to meet the eligibility criteria for the study (section 6) at the next check (visit 3, 6 or 7), they will be withdrawn if the visit cannot be postponed. Participants who receive at least one dose of IMP or endotoxin will be followed up by telephone 10-14 days after withdrawal.

Other anti-inflammatory/immunomodulatory drugs

Oral, topical or inhaled corticosteroids; disease-modifying anti-rheumatic drugs; immunosuppressants; chemotherapy drugs; oral or topical antihistamines: In the case of a participant requiring regular treatment with these agents, if they fail to meet the eligibility criteria for the study (section 6) at the next check (visit 3, 6 or 7), they will be withdrawn if the visit cannot be postponed Participants who receive at least one dose of IMP or endotoxin will be followed up by telephone 10-14 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. If a requirement for regular diuretic treatment develops during the study, a participant who fails to meet the eligibility criteria for the study (section 6) at the next check (visit 3, 6 or 7), will be withdrawn if the visit cannot be postponed . Those who receive at least one dose of IMP or endotoxin will be followed up by telephone 10-14 days after withdrawal.

ACE inhibitors/Angiotensin receptor blockers

ACE inhibitors and angiotensin receptor blockers can affect renal prostaglandin production. If a participant fails to meet the eligibility criteria for the study (section 6) at the next check (visit 3, 6 or 7), they will be withdrawn if the visit cannot be postponed but followed up by telephone at 10-14 days after withdrawal.

Parenteral anticoagulants

In the event of an indication for parenteral anticoagulation developing, the participant will be withdrawn from the study but participants who receive at least one dose of IMP or endotoxin will be followed up by telephone 10-14 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 or endotoxin will be followed up by telephone 10-14 days after withdrawal.

Oral anticoagulants

The use of oral anticoagulants (e.g. warfarin, apixaban, rivaroxaban, dabigatran) in combination with study medication is prohibited. If oral anticoagulation is considered essential during the intended course of the study, the participant will be withdrawn from the study. Participants who receive at least one dose of IMP or endotoxin will be followed up by telephone 10-14 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 or endotoxin will be followed up by telephone 10-14 days after withdrawal.

Other drugs

If regular treatment with any other prescribed or over-the-counter systemic or topical medication becomes required after randomisation, if a participant fails to meet the eligibility criteria for the study (section 6) at the next check (visit 3, 6 or 7), they will be withdrawn if the visit cannot be postponed.

8.10 Trial restrictions

Whilst undergoing sampling during visits 3 and 7, subjects will take a light breakfast before 8 am then remain nil by mouth until 2 hours post-endotoxin.

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 or endotoxin will be followed up by telephone 10-14 days after withdrawal.

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

Intercurrent illness

In the event of a participant developing an intercurrent illness after randomisation, for example a viral upper respiratory tract infection, medication periods or endotoxin injections may be postponed, at the discretion of the investigator, until resolution of the illness. A file note should be generated and placed in the trial master file.

8.11 Assessment of compliance with treatment

Compliance will be assessed at the end of each treatment period by counting the remaining sachets of aspirin (aspirin lysine) returned by the participant, and by direct observation of the participant taking the loading dose of ticagrelor and last dose of aspirin in each period, where applicable. Sachet 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)

Although not a medicinal product, in this study we are additionally administering **sterile bacterial endotoxin**, 2 ng/kg intravenously. As a challenge agent, this is classified as a NIMP, as per European Commission Guidance Document SANCO/C/8/SF/cg/a.5.001(2011)332855. This will be reconstituted with water for injection as per the manufacturer's instructions. Endotoxin specifically designed for human challenge studies will be obtained from Dr Anthony Suffredini, National Institutes of Health, Bethesda, USA. Endotoxin will be securely stored separately in cold storage within the Cardiovascular Research Unit, Northern General Hospital. The temperature of the storage environment will be monitored by recording a daily maximum and minimum temperature log (with the exception of weekends). The allowable temperature range will be 2 to 8 degrees Celsius. An accountability log to track each vial will be kept in the trial master file. Endotoxin will be prescribed by a medically-qualified investigator on a Sheffield Teaching Hospitals Drug Administration Record. Administration will be recorded on the same chart.

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 Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out in the reference safety information: • SmPC for aspirin (aspirin lysine) • SmPC for ticagrelor

9.2 Operational definitions for (S)AEs

Adverse events and reactions will be recorded and reported from randomisation to 10 days after visit 3 (first endotoxin injection day), and from visit 6 (start of period 2) to 10 days after visit 7 (second endotoxin injection day). Thus, adverse events occurring in the break between the first and second medication periods, excepting those originating in the 10 days after endotoxin injection, will not be recorded or reported.

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, with the exception of the following events which will not be reported, as they can be expected as a normal response to endotoxin:

- Abnormal body temperature, excepting if >39°C and <34°C, unless this persists at 6 hours post-endotoxin or meets the criteria for reporting as an SAE.
- Change in pulse rate, excepting pulse rates of >140 bpm and <45 bpm or if meeting the criteria for SAE reporting.
- Change (increase or decrease) in systolic or diastolic blood pressure from baseline of <40 mmHg unless meeting the criteria for SAE reporting.
- Self-limiting symptoms wholly due to endotoxaemia, for example fever, rigors, general malaise, nausea, vomiting or headache, at the discretion of the investigator, unless meeting the criteria for SAE reporting.
- Alterations in leukocyte (or subset) counts, platelet count or C-reactive protein unless meeting the criteria for SAE reporting.

The investigators will assess the relatedness of adverse events to the IMPs (aspirin [aspirin lysine], ticagrelor) and NIMP (endotoxin).

9.3 Recording and reporting of SAEs, SARs AND SUSARs

SAEs/SUSARs will be recorded and reported from randomisation to 10 days after visit 3 (first endotoxin injection day), and from visit 6 (start of period 2) to 10 days after visit 7 (second endotoxin injection day). Thus, adverse events occurring in the break between the first and second medication periods, excepting those originating in the 10 days after endotoxin injection, will not be recorded or reported.

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.

SAEs related to the NIMP (endotoxin) will be reported by the investigators to the sponsor, but further forwarding to other agencies will not be required, as per European Directive 2011/C 172/01. NIMP-related SAEs will be evaluated by the sponsor and investigator team with regard to ongoing safety implications. If there are ongoing safety implications for the trial then these will be addressed via urgent safety measure, substantial amendment or termination of the trial.

If, on adjudication by the investigators, there is a possibility that an SAE is related to interaction between the NIMP and at least one of the IMPs, the process will be followed for reporting this as an IMP-related event, including any required onward notification by the sponsor concerning the relevant IMP.

If an SAE is judged as possibly related to both NIMP and an IMP then it will be assessed with regard to expectedness with regard to the IMP and reported onwards as a SUSAR if it is judged to be unexpected in relation to the IMP

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. Using medical judgement in assigning seriousness and causality and providing an opinion on whether the event/reaction was anticipated using the Reference Safety Information approved for the trial.
4. 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.
5. 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).

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

1. Collection and verification of AEs, ARs, SAEs, SARs and SUSARs according to the trial protocol.

2. Expedited reporting of SUSARs to the Competent Authority (MHRA) and REC within required timelines.
3. Checking for (annually) and notifying PIs of updates to the Reference Safety Information for the trial.
4. 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, endotoxin administration or an unrelated event.

9.6 Pregnancy reporting

- Pregnancy is not expected to occur as women of childbearing potential 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, ticagrelor 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

In our group's previous study of endotoxaemia in 30 participants receiving ticagrelor, clopidogrel or placebo, we saw, after log transformation, a mean 2 hour post-endotoxin plasma TNF-α 1.88 pg/ml with a standard deviation of 0.410 pg/ml.

There is no available raw data and no reliable estimate of the means or standard deviations of the log transformed TNF-α values for any of the three aspirin doses using the same methods. However, a study using a different endotoxin regimen has shown that aspirin 80 mg OD (which is not expected to be significantly different in effect to 75 mg OD) increased peak plasma TNF-α by around 40% compared to those receiving no drug.

We have a clear hypothesis that increasing aspirin dose increases TNF-α, therefore a one-sided test will suffice.

We estimate that clinically-relevant differences in plasma TNF-α at 2 hours post-endotoxin between the dosing regimens might be a 20% increase with 20 mg BD vs. no aspirin, 40% increase with 75 mg OD vs. no aspirin and 60% with 300 mg OD vs. no aspirin, based on the raw data.

These assumptions provide the following sample size estimate:

Test significance level, α	0.050
Number of groups, G	4
Variance of means, $V=S(m_i-m)^2 / G$	0.031
Common standard deviation, s	0.410
Effect size, $D^2 = V/s^2$	0.1828
Power (%)	80
n per group	16

Therefore, when the sample size in each of the 4 groups is 16, a one-way analysis of variance will have 80% power to detect at the 0.050 level a difference in means characterised by a Variance of means, $V=\Sigma(\mu_i-\mu)^2 / G$ of 0.031, assuming that the common standard deviation is 0.410.

To allow for up to 10% drop-out, the sample size per group will be 18. Drop-outs will not be replaced but it is expected that there will be at least 16 per group after withdrawals.

This sample size calculation was performed by Mrs Kathleen Baster CStat, Senior Consultant, Statistical Services Unit, University of Sheffield, to whom the investigators are grateful.

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

At visit 2

- TNF-α, IL-6, serum TXB_2, urine PGI-M, platelet aggregation responses to arachidonic acid, ADP and collagen
- Bleeding time

Categorical data will be reported as proportions and percentages. Differences between the groups will be assessed using Fisher's exact contingency test. Continuous data will be reported as mean and standard deviation if normally distributed otherwise median and interquartile range. Differences between the parallel groups will be assessed with ANOVA, with Bonferroni pairwise comparisons made if any ANOVA reaches $p<0.05$.

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 plasma TNF-α at 2 hours following endotoxin administration assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, without ticagrelor) by one-way ANOVA with treatment as a factor.

10.3.3 Secondary outcome analysis

Secondary analyses will be as follows:

Between aspirin dose regimens

1. Plasma TNF-α over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, without ticagrelor) by two-way mixed ANOVA with treatment as a between-subjects factor and timepoint as a within-subjects factor.

2. Plasma IL-6 at 2 hours following endotoxin administration assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, without ticagrelor) by one-way ANOVA with treatment as a factor.
3. Plasma IL-6 over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, without ticagrelor) by two-way mixed ANOVA with treatment as a between-subjects factor and timepoint as a within-subjects factor.

4. Change in serum CRP over time following endotoxin administration (measured at 0 and 6 hours post-endotoxin) assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, without ticagrelor) by one-way ANOVA.

5. Serum TXB_2 over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, without ticagrelor) by two-way mixed ANOVA with treatment as a between-subjects factor and timepoint as a within-subjects factor.

6. Urinary PGI-M (measured at 0, 2, 4 and 6 hours, each adjusted for [divided by] urinary creatinine) over time following endotoxin administration assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, without ticagrelor) by two-way mixed ANOVA with treatment as a between-subjects factor and timepoint as a within-subjects factor.

7. Serum PGE_2 over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, without ticagrelor) by two-way mixed ANOVA with treatment as a between-subjects factor and timepoint as a within-subjects factor.

8. Circulating leukocyte count over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, without ticagrelor) by two-way mixed ANOVA with treatment as a between-subjects factor and timepoint as a within-subjects factor.

9. AUC of the graph of bleeding time following endotoxin administration (measured at 0 and 6 hours post-endotoxin) assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, without ticagrelor) by one-way ANOVA.

10. AUC of the graphs of platelet aggregation to arachidonic acid (0.3 and 1 mmol/L), collagen (4 and 16 μg/ml) and ADP (20 μmol/L) over time following endotoxin administration (measured at 0 and 6 hours post-endotoxin) assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, without ticagrelor) by one-way ANOVA.

Between DAPT regimens:

11. Plasma TNF-α at 2 hours following endotoxin administration assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, given in combination with ticagrelor) by one-way ANOVA with treatment as a factor.

12. Plasma TNF-α over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, given in combination with ticagrelor) by two-way mixed ANOVA with treatment as a between-subjects factor and timepoint as a within-subjects factor.

13. Plasma IL-6 at 2 hours following endotoxin administration assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, given in combination with ticagrelor) by one-way ANOVA with treatment as a factor.

14. Plasma IL-6 over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, given in combination with ticagrelor) by two-way mixed ANOVA with treatment as a between-subjects factor and timepoint as a within-subjects factor.

15. Change in serum CRP over time following endotoxin administration (measured at 0 and 6 hours post-endotoxin) assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, given in combination with ticagrelor) by one-way ANOVA.

16. Serum TXB_2 over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, given in combination with ticagrelor) by two-way mixed ANOVA with treatment as a between-subjects factor and timepoint as a within-subjects factor.

17. Urinary PGI-M (measured at 0, 2, 4 and 6 hours, each adjusted for [divided by] urinary creatinine) over time following endotoxin administration assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, given in combination with ticagrelor) by two-way mixed ANOVA with treatment as a between-subjects factor and timepoint as a within-subjects factor.

18. Serum PGE_2 over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, given in combination with ticagrelor) by two-way mixed ANOVA with treatment as a between-subjects factor and timepoint as a within-subjects factor.

19. Circulating leukocyte count over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) assessed between aspirin groups (control vs. 20 mg BD vs.

75 mg OD vs. 300 mg OD, given in combination with ticagrelor) by two-way mixed ANOVA with treatment as a between-subjects factor and timepoint as a within-subjects factor.

20. AUC of the graph of bleeding time following endotoxin administration (measured at 0 and 3 hours post-endotoxin) assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, given in combination with ticagrelor) by one-way mixed ANOVA.

21. AUC of the graphs of platelet aggregation to arachidonic acid (0.3 and 1 mmol/L), collagen (4 and 16 μg/ml) and ADP (20 μmol/L) over time following endotoxin administration (measured at 0 and 3 hours post-endotoxin) assessed between aspirin groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, given in combination with ticagrelor) by one-way ANOVA.

To compare aspirin monotherapy with aspirin and ticagrelor in combination:

1. Plasma TNF-α at 2 hours following endotoxin administration) compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD) by paired t-tests.

2. AUC of the graph of plasma TNF-α over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD) by paired t-tests.

3. Plasma IL-6 at 2 hours following endotoxin administration, compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD) by paired t-tests.

4. AUC of the graph of plasma IL-6 over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD) by paired t-tests.

5. Change in serum CRP over time following endotoxin administration (measured at 0 and 6 hours post-endotoxin) compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD) by paired t-tests.

6. AUC of the graph of serum TXB_2 over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD) by paired t-tests.

7. AUC of the graph of urinary PGI-M (measured at 0, 2, 4 and 6 hours, each adjusted for [divided by] urinary creatinine) over time following endotoxin administration compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD) by paired t-tests.

8. AUC of the graph of serum PGE_2 over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) compared between each aspirin regimen (control, 20 mg

BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD) by paired t-tests.

9. AUC of the graph of circulating leukocyte count over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD) by paired t-tests.

10. AUC of the graph of bleeding time following endotoxin administration (measured at 0 and 3 hours post-endotoxin) compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD) by paired t-tests.

11. AUC of the graphs of platelet aggregation to arachidonic acid (0.3 and 1 mmol/L), collagen (4 and 16 μg/ml) and ADP (20 μmol/L) over time following endotoxin administration (measured at 0 and 3 hours post-endotoxin) compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD) by paired t-tests.

And to model the effects of all variables (considering parallel groups):

12. Plasma TNF-α over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD)) with a mixed three-way ANOVA with aspirin-dose and presence or absence of ticagrelor as between-subjects factors and timepoint as a within-subject factor.

13. Plasma IL-6 over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD) with a mixed three-way ANOVA with aspirin-dose and presence or absence of ticagrelor as between-subjects factors and timepoint as a within-subject factor.

14. Change in serum CRP over time following endotoxin administration (measured at 0 and 6 hours post-endotoxin) compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD) with a mixed three-way ANOVA with aspirin-dose and presence or absence of ticagrelor as between-subjects factors and timepoint as a within-subject factor.

15. Serum TXB_2 over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD)) with a mixed three-way ANOVA with aspirin-dose and presence or absence of ticagrelor as between-subjects factors and timepoint as a within-subject factor.

16. Urinary PGI-M (measured at 0, 2, 4 and 6 hours, each adjusted for [divided by] urinary creatinine) over time following endotoxin administration compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD) with a mixed three-way ANOVA with aspirin-dose and presence or absence of ticagrelor as between-subjects factors and timepoint as a within-subject factor.

17. Serum PGE_2 over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD) with a mixed three-way ANOVA with aspirin-dose and presence or absence of ticagrelor as between-subjects factors and timepoint as a within-subject factor.

18. Circulating leukocyte count over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD)) with a mixed three-way ANOVA with aspirin-dose and presence or absence of ticagrelor as between-subjects factors and timepoint as a within-subject factor.

19. Bleeding time following endotoxin administration (measured at 0 and 3 hours post-endotoxin) compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD) with a mixed three-way ANOVA with aspirin-dose and presence or absence of ticagrelor as between-subjects factors and timepoint as a within-subject factor.

20. Platelet aggregation to arachidonic acid (0.3 and 1 mmol/L), collagen (4 and 16 μg/ml) and ADP (20 μmol/L) over time following endotoxin administration (measured at 0 and 3 hours post-endotoxin) compared between each aspirin regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each without ticagrelor) and each corresponding DAPT regimen (control, 20 mg BD, 75 mg OD, 300 mg OD, each with ticagrelor 90 mg BD) with a mixed three-way ANOVA with aspirin-dose and presence or absence of ticagrelor as between-subjects factors and timepoint as a within-subject factor.

For all endpoints (primary and secondary) tested with ANOVA, pairwise comparisons with Bonferroni correction will be used to explore relationships further where the prespecified test suggests significant differences between the groups ($p<0.05$).

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

After discussion between the investigators and the Sponsor, face-to-face participant study visits were halted on 17th March 2020 due to the unprecedented circumstances arising during the coronavirus disease 2019 (Covid-19) pandemic. It was considered by the investigators that the results of the study may have important implications for the management of cardiovascular disease patients who contracted Covid-19 in terms of optimal antiplatelet medication, in view of the morbidity and mortality in Covid-19 associated with dysregulated inflammatory response. It was noted that the recruitment and study activities to date had reached the level that had originally been planned for the study, as per the application funded by the British Heart Foundation. Given the uncertainty regarding restarting the trial, the need to preserve healthcare resources and to limit face-to-face encounters, the investigators and Sponsor decided that an interim analysis should be performed.

Sample size for interim analysis

Data from all participants who have completed visit 3 (first endotoxin day) will be included in the interim analysis. This is expected to include 46 participants who have completed visit 3, 37 of whom have completed the whole study giving a total of 83 endotoxin day visits, anticipated to be split into approximately equal numbers receiving each of the eight study treatment regimens.

Statistical procedure for interim analysis

The primary endpoint of the interim analysis will be plasma TNF-α measured at 2 hours after endotoxin administration assessed between aspirin treatment groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, without ticagrelor) using one-way ANOVA.

Should this demonstrate a significant difference between the groups ($p<0.05$), the following pre-specified pairwise comparisons will be performed, in hierarchical fashion:

300 mg OD vs. no aspirin

then, if significant ($p<0.05$)

75 mg OD vs. no aspirin

then, if significant ($p<0.05$)

300 mg OD vs 20 mg BD

then, if significant ($p<0.05$)

75 mg OD vs 20 mg BD

then, if significant ($p<0.05$)

20 mg BD vs no aspirin

Other analyses will be exploratory.

Criteria for premature termination

The trial shall be prematurely terminated if the primary interim analysis demonstrates a statistically significant ($p<0.05$) effect of aspirin dosing on plasma TNF-α two hours after endotoxin injection.

Additionally, continuing the study will be considered futile, and therefore discontinued, if the lower limit of the 95 % confidence interval of the mean change in TNF-α at 2 hours after endotoxin from the 300 mg aspirin OD group to no aspirin group is less than (more negative than) -2000 pg/mL.

Should neither of these criteria be met, the investigators will meet to review the results of the interim analysis and, in discussion with the Sponsor, decide on whether to continue the study, taking into account feasibility, including consideration of local and national restrictions.

Secondary analyses to be carried out in the event of premature termination

If the trial is prematurely terminated, data concerning other endpoints will then be analysed as follows, using methods taking best into account the cases of participant data missing from one period:

1. Plasma TNF-α over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) assessed between treatment groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, with or without ticagrelor) using a mixed effect linear model with patient as a random effect, aspirin dose, presence or absence of ticagrelor treatment and treatment period (first or second) as fixed effects, and baseline value as covariate.

2. Plasma IL-6 over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) assessed between treatment groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, with or without ticagrelor) using a mixed effect linear model with patient as a random effect, aspirin dose, presence or absence of ticagrelor treatment and treatment period (first or second) as fixed effects, and baseline value as covariate.

3. Change in serum CRP over time following endotoxin administration (measured at 0 and 6 hours post-endotoxin) assessed between treatment groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, with or without ticagrelor) using a mixed effect linear model with patient as a random effect, aspirin dose, presence or absence of ticagrelor treatment and treatment period (first or second) as fixed effects, and baseline value as covariate.

4. Serum TXB_2 over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) assessed between treatment groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, with or without ticagrelor) using a mixed effect linear model with patient as a random effect, aspirin dose, presence or absence of ticagrelor treatment and treatment period (first or second) as fixed effects, and baseline value as covariate.

5. Urinary PGI-M (measured at 0, 2, 4 and 6 hours, each adjusted for [divided by] urinary creatinine) over time following endotoxin administration, assessed between treatment groups (control vs. 20

mg BD vs. 75 mg OD vs. 300 mg OD, with or without ticagrelor) using a mixed effect linear model with patient as a random effect, aspirin dose, presence or absence of ticagrelor treatment and treatment period (first or second) as fixed effects, and baseline value as covariate.

6. Serum PGE_2 over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) assessed between treatment groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, with or without ticagrelor) using a mixed effect linear model with patient as a random effect, aspirin dose, presence or absence of ticagrelor treatment and treatment period (first or second) as fixed effects, and baseline value as covariate.

7. Circulating leukocyte count over time following endotoxin administration (measured at 0, 0.5, 1, 1.5, 2, 3, 4 and 6 hours post-endotoxin) assessed between treatment groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, with or without ticagrelor) using a mixed effect linear model with patient as a random effect, aspirin dose, presence or absence of ticagrelor treatment and treatment period (first or second) as fixed effects, and baseline values as covariate.

8. Bleeding time following endotoxin administration (measured at 0 and 3 hours post-endotoxin) assessed between treatment groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, with or without ticagrelor) using a mixed effect linear model with patient as a random effect, aspirin dose, presence or absence of ticagrelor treatment and treatment period (first or second) as fixed effects, and baseline values as covariate.

9. Platelet aggregation to arachidonic acid (0.3 and 1 mmol/L), collagen (4 and 16 μg/ml) and ADP (20 μmol/L) over time following endotoxin administration (measured at 0 and 6 hours post-endotoxin) assessed between treatment groups (control vs. 20 mg BD vs. 75 mg OD vs. 300 mg OD, with or without ticagrelor) using a mixed effect linear model with patient as a random effect, aspirin dose and presence or absence of ticagrelor treatment and treatment period as fixed effects, and baseline values as covariate.

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 two periods and who complete both endotoxin injection days.
- 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 trial drug or one dose of intravenous endotoxin.

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). 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 SOPs for the use of the CRF and database, maintain an audit trail of data changes ensuring that there is no deletion of entered data, maintain a security system to protect against unauthorized access, 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 at least 15 years, at which point storage arrangements will be reviewed, in electronic format on University of Sheffield file storage systems. This will be stated in the PIS.

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, accuracy, and timeliness of data collection.
- 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 at the site.
- 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 British Heart Foundation, who also reviewed the statistical aspects.

13.3 Public and Patient Involvement

Members of the Sheffield Cardiovascular Patient Panel have been involved in reviewing the design of the study and key participant documents during development of this protocol and associated documentation.

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, and PlaqueTec; consultancy fees from Actelion, AstraZeneca, Avacta, Bayer, Bristol Myers Squibb, Novartis, PlaqueTec, Haemonetics and Thromboserin; speaker fees from AstraZeneca, and Bayer. 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 DISSEMINIATION 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 British Heart Foundation 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.

15 REFERENCES

- 1. Jackson, S.P., *Arterial thrombosis--insidious, unpredictable and deadly.* Nat Med, 2011. **17**(11): p. 1423-36.
- 2. Luc, G., et al., *C-reactive protein, interleukin-6, and fibrinogen as predictors of coronary heart disease: the PRIME Study.* Arterioscler Thromb Vasc Biol, 2003. **23**(7): p. 1255-61.
- 3. Roffi, M., et al., *2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation.* Eur Heart J, 2015. **37**: p. 267-315.
- 4. Steg, P.G., et al., *ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation.* Eur Heart J, 2012. **33**(20): p. 2569-619.
- 5. Wallentin, L., et al., *Ticagrelor versus clopidogrel in patients with acute coronary syndromes.* N Engl J Med, 2009. **361**(11): p. 1045-57.
- 6. Ridker, P.M., et al., *Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease.* N Engl J Med, 2017. **377**(12): p. 1119-1131.
- 7. Thomas, M.R., et al., *Platelet P2Y12 Inhibitors Reduce Systemic Inflammation and Its Prothrombotic Effects in an Experimental Human Model.* Arterioscler Thromb Vasc Biol, 2015. **35**(12): p. 2562-70.
- 8. Iqbal, A., et al., *Hypoglycaemia increases platelet reactivity and promotes formation of proatherogenic*
- *platelet-leucocyte aggregates.* Diabetologia, 2017. **60**: p. S532.
- 9. Patrono, C., et al., *Antiplatelet Agents for the Treatment and Prevention of Coronary Atherothrombosis.* J Am Coll Cardiol, 2017. **70**(14): p. 1760-1776.
- 10. Patrono, C., *Aspirin as an antiplatelet drug.* N Engl J Med, 1994. **330**(18): p. 1287-94.
- 11. investigators, I.-. *Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group.* Lancet, 1988. **2**(8607): p. 349-60.
- 12. Belch, J., et al., *The prevention of progression of arterial disease and diabetes (POPADAD) trial: factorial randomised placebo controlled trial of aspirin and antioxidants in patients with diabetes and asymptomatic peripheral arterial disease.* BMJ, 2008. **337**.
- 13. Pignone, M., et al., *Aspirin for Primary Prevention of Cardiovascular Events in People With Diabetes: A position statement of the American Diabetes Association, a scientific statement of the American Heart Association, and an expert consensus document of the American College of Cardiology Foundation.* Diabetes Care, 2010. **33**(6): p. 1395-402.
- 14. Ogawa, H., et al., *Low-dose aspirin for primary prevention of atherosclerotic events in patients with type 2 diabetes: a randomized controlled trial.* Jama, 2008. **300**(18): p. 2134-41.

- 15. Ikeda, Y., et al., *Low-dose aspirin for primary prevention of cardiovascular events in Japanese patients 60 years or older with atherosclerotic risk factors: a randomized clinical trial.* Jama, 2014. **312**(23): p. 2510-20.
- 16. Gaziano, J.M., et al., *Use of aspirin to reduce risk of initial vascular events in patients at moderate risk of cardiovascular disease (ARRIVE): a randomised, double-blind, placebo-controlled trial.* Lancet, 2018.
- 17. Paul-Clark, M.J., et al., *15-epi-lipoxin A4-mediated induction of nitric oxide explains how aspirin inhibits acute inflammation.* J Exp Med, 2004. **200**(1): p. 69-78.
- 18. Kiers, D., et al., *A randomised trial on the effect of anti-platelet therapy on the systemic inflammatory response in human endotoxaemia.* Thromb Haemost, 2017. **117**(9): p. 1798-1807.
- 19. McAdam, B.F., et al., *Effect of regulated expression of human cyclooxygenase isoforms on eicosanoid and isoeicosanoid production in inflammation.* J Clin Invest, 2000. **105**(10): p. 1473-82.
- 20. Birrell, M.A., et al., *Anti-inflammatory effects of PGE2 in the lung: role of the EP4 receptor subtype.* Thorax, 2015. **70**(8): p. 740-7.
- 21. Na, Y.R., et al., *Endogenous prostaglandin E2 potentiates anti-inflammatory phenotype of macrophage through the CREB-C/EBP-beta cascade.* Eur J Immunol, 2015. **45**(9): p. 2661-71.
- 22. Chow, J.C., et al., *Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction.* J Biol Chem, 1999. **274**(16): p. 10689-92.
- 23. Brasier, A.R., *The nuclear factor-kappaB-interleukin-6 signalling pathway mediating vascular inflammation.* Cardiovasc Res, 2010. **86**(2): p. 211-8.
- 24. Degraaf, A.J., et al., *Prostaglandin E2 reduces Toll-like receptor 4 expression in alveolar macrophages by inhibition of translation.* Am J Respir Cell Mol Biol, 2014. **51**(2): p. 242-50.
- 25. Lee, K.M. and S.Y. Seong, *Partial role of TLR4 as a receptor responding to damage-associated molecular pattern.* Immunol Lett, 2009. **125**(1): p. 31-9.
- 26. Smiley, S.T., J.A. King, and W.W. Hancock, *Fibrinogen stimulates macrophage chemokine secretion through toll-like receptor 4.* J Immunol, 2001. **167**(5): p. 2887-94.
- 27. Smeeth, L., et al., *Risk of myocardial infarction and stroke after acute infection or vaccination.* N Engl J Med, 2004. **351**(25): p. 2611-8.
- 28. Morris, T., et al., *Effects of low-dose aspirin on acute inflammatory responses in humans.* J Immunol, 2009. **183**(3): p. 2089-96.
- 29. Layne, K., et al., *Anti-platelet drugs attenuate the expansion of circulating CD14highCD16+ monocytes under pro-inflammatory conditions.* Cardiovasc Res, 2016. **111**(1): p. 26-33.
- 30. Serezani, C.H., et al., *Leukotriene B4 amplifies NF-kappaB activation in mouse macrophages by reducing SOCS1 inhibition of MyD88 expression.* J Clin Invest, 2011. **121**(2): p. 671-82.
- 31. Vranckx, P., et al., *Ticagrelor plus aspirin for 1 month, followed by ticagrelor monotherapy for 23 months vs aspirin plus clopidogrel or ticagrelor for 12 months, followed by aspirin monotherapy for 12 months after implantation of a drug-eluting stent: a multicentre, open-label, randomised superiority trial.* Lancet, 2018.
- 32. Baber, U., et al., *Ticagrelor with aspirin or alone in high-risk patients after coronary intervention: Rationale and design of the TWILIGHT study.* Am Heart J, 2016. **182**: p. 125-134.
- 33. Teng, R., J. Maya, and K. Butler, *Evaluation of the pharmacokinetics and pharmacodynamics of ticagrelor co-administered with aspirin in healthy volunteers.* Platelets, 2013. **24**(8): p. 615-624.
- 34. Scavone, M., et al., *Inhibition of the platelet P2Y12 receptor for adenosine diphosphate does not impair the capacity of platelet to synthesize thromboxane A2.* Eur Heart J, 2015.
- 35. Mahaffey, K.W., et al., *Ticagrelor Compared With Clopidogrel by Geographic Region in the Platelet Inhibition and Patient Outcomes (PLATO) Trial.* Circulation, 2011. **124**(5): p. 544-554.

- 36. Parker, W., et al., *Ultra-low-dose twice-daily aspirin improves hemostasis and maintains platelet inhibition in acute coronary syndrome patients receiving ticagrelor.* Journal of the American College of Cardiology, 2018. **71**(11 Supplement): p. A30.
- 37. *Brilique 90 mg film coated tablets - Summary of Product Characteristics (SmPC) - (eMC).* 2018 [cited 2018 14 May 2018]; Available from: https://www.medicines.org.uk/emc/product/5767/smpc.
- 38. Suffredini, A.F., H.D. Hochstein, and F.G. McMahon, *Dose-related inflammatory effects of intravenous endotoxin in humans: evaluation of a new clinical lot of Escherichia coli O:113 endotoxin.* J Infect Dis, 1999. **179**(5): p. 1278-82.
- 39. Rittig, N., et al., *Hormone and Cytokine Responses to Repeated Endotoxin Exposures-No Evidence of Endotoxin Tolerance After 5 Weeks in Humans.* Shock, 2015. **44**(1): p. 32-5.

APPENDIX 1 Sample collection plan

Visit	Visit description	Timepoint within visit	Safety (FBC)	Safety (Chemistry)	Safety (Clotting screen)	DNA/RNA for storage	Plasma for cytokines/storage	Serum for prostanoids/CRP /storage	Plasma for LTA	Blood for white cell count and subsets/storage	Total for timepoint	Urine	Bleeding time
		Tube type ⇨	EDTA	SST	Citrate	EDTA	Citrate	SST	Citrate	Citrate			
			Whole blood to draw (ml)										
1	Enrolment		4	4	4.5						12.5	✓(Dip only)	
2	Randomisation					4	9	5	9	4.5	27	✓	✓
3	Endotoxin day 1	Baseline (pre drug dose)	4	4	4.5		4.5	5	9	4.5	35.5	✓	✓
		Post drug dose/pre endotoxin					4.5	5	9	4.5	23		
		30 mins post endotoxin					4.5	5		4.5	14		
		1 hour post endotoxin					4.5	5		4.5	14	✓	
		1.5 hours post endotoxin					4.5	5		4.5	14		
		2 hours post endotoxin					9	5		9	23	✓	
		3 hours post endotoxin					4.5	5	9	4.5	23		✓
		4 hours post endotoxin					4.5	5		4.5	14	✓	
		6 hours post endotoxin					4.5	5		4.5	14	✓	
4	(Telephone call only)										0		
5	(Telephone call only)												
6	Start of period 2		4	4	4.5						12.5		
7	Endotoxin day 2	Baseline (pre drug dose)	4	4	4.5		4.5	5	9	4.5	35.5	✓	✓
		Post drug dose/pre endotoxin					4.5	5	9	4.5	23		
		30 mins post endotoxin					4.5	5		4.5	14		
		1 hour post endotoxin					4.5	5		4.5	14	✓	
		1.5 hours post endotoxin					4.5	5		4.5	14		
		2 hours post endotoxin					9	5		9	23	✓	
		3 hours post endotoxin					4.5	5	9	4.5	23		✓
		4 hours post endotoxin					4.5	5		4.5	14	✓	
		6 hours post endotoxin					4.5	5		4.5	14	✓	
8	(Telephone call only)										0		
9	(Telephone call only)										0		
		TOTAL	16	16	18	4	99	95	63	94.5	**401**		

EDTA, Ethylenediaminetetraacetic acid; LTA, light transmittance aggregometry; SST, serum separator tube. See main text of protocol for other abbreviations.