A RandomisEd trial of two diFferent antIplatelet strategies in patieNts with acutE coronary syndromes planned for Coronary Artery Bypass Graft surgery - the REFINE CABG study

Clinical Study Protocol

RESEARCH REFERENCE NUMBERS
STH21981

TRIAL REGISTRY NUMBER

ISRCTN registry: ISRCTN17514416

OTHER RESEARCH REFERENCE NUMBERS

MHRA: CTA 21304/0277/001-0001
IRAS ID: 1005625

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: Date:

..../...../.....

Name (please print):

.....

Position:

.....

Chief Investigator:

Date:

Signature: ...../...../.....

.....

Name: (please print): Professor Robert Storey

KEY TRIAL CONTACTS

Chief and 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 Email
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Perfusionist	Michelle Jackson Chief Perfusionist Northern General Hospital Herries Road, Sheffield S5 7AU Email:

ii. LIST OF ABBREVATIONS

AA	Arachidonic Acid
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
BMI	Body Mass Index
CI	Chief Investigator
COX	Cyclo-oxygenase
CPB	Cardiopulmonary bypass
CRF	Case Report Form
CTA	Clinical Trial Authorisation
CV	Cardiovascular
CYP	Cytochrome P450
DSUR	Development safety update report
ECG	Electrocardiography
EudraCT	European Clinical Trials Database
GCP	Good Clinical Practice
GP	General Practitioner
HRA	Health Research Authority
ICF	Informed Consent Form
IL-6	Interleukin 6
IMP	Investigation Medicinal Product
L	Litre
mg	Milligrams
MHRA	Medicines and Healthcare Regulatory Authority
MI	Myocardial Infarction
mL	Millilitres
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NR	Not Recorded
NSAID	Non-Steroidal Anti-Inflammatory Drug
OD	Once-daily
$P2Y_{12}$	Platelet adenosine diphosphate receptor
PI	Principal Investigator
PIS	Patient Information Sheet
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
TMF	Trial Master File
TNF-α	Tumour Necrosis Factor α
TXA_2	Thromboxane A_2
µg	Micrograms
UK	United Kingdom
µmol	Micromoles
WOCBP	Women of child-bearing potential

iii. TRIAL SUMMARY

Trial Title	A RandomisEd trial of two diFferent antIplatelet strategies in patieNts with acutE coronary syndromes planned for Coronary Artery Bypass Graft surgery - the REFINE CABG study
Internal ref. no.	STH21981
Clinical Phase	Phase IV
Trial Design	Open label, single-centre, randomised clinical study of two independent parallel groups
Trial Participants	Participants with acute coronary syndromes aged 18 years or over, male or female (women of child-bearing potential with a negative pregnancy test at screening), planned for urgent coronary artery bypass graft surgery with cardiopulmonary bypass (CPB) who are currently receiving aspirin 75mg od and are currently receiving or have previously received during the current admission ticagrelor 90mg bd
Planned Sample Size	40

Experimental group	Patients will receive ticagrelor and aspirin until the morning of either the day before CABG surgery (initial study design for first 10 randomised patients undergoing CABG surgery) or two days before CABG surgery (study design after 12 May 2023 for subsequent 30 randomised patients undergoing CABG surgery) and will then discontinue these. Patients will receive treatment with the CytoSorb device during surgery to reduce plasma ticagrelor levels.	
Standard-of-care group	Patients will discontinue ticagrelor approximately 5 days before CABG surgery and will continue to receive aspirin up to the day of CABG surgery.	
Treatment duration for ticagrelor	Experimental group: From 0 days up to 20 days; standard-of-care group from 3 days up to 18 days	
Treatment duration for aspirin	Experimental group: from 0 days up to 20 days; standard-of-care group: from 3 days up to 21 days	
Follow up duration	Until 30 days after CABG surgery	
Planned Trial Period	August 2022-March 2024	
	Summary Objectives	**Summary Outcome Measures**
Primary	Assess platelet reactivity immediately following CPB	Platelet aggregation induced by collagen 4 ug/mL assessed by light transmittance aggregometry (LTA)
Secondary and Tertiary	Assess effects of strategy and surgery on platelet function	LTA responses to collagen, arachidonic acid, adenosine diphosphate (ADP), 5-hydroxytryptamine + epinephrine, and thrombin-receptor-activating peptide (TRAP) VerifyNow $P2Y_{12}$ assay Multiplate ADPtest and TRAPtest VASP phosphorylation assay ADP-induced platelet P-selectin expression Serum thromboxane B_2
	Assess inflammatory response to surgery	Plasma interleukin-6 Plasma tumour necrosis factor α Circulating leukocyte counts plus subsets and platelet-leukocyte aggregates Neutrophil and monocyte CD11b expression
	Assess effects of surgery on coagulation and haemostasis	Fibrin clot dynamics Plasma D-dimer levels

		Bleeding time Post-surgery chest tube drainage
	Assess ticagrelor pharmacokinetics	Plasma ticagrelor concentration Plasma ticagrelor active metabolite concentration
	Assess effects on length of stay	Total length of hospital stay Time from coronary angiography to CABG surgery, Time from randomisation to CABG surgery Time from randomisation to hospital discharge Time on intensive care unit
Investigational Medicinal Product(s)	1. Ticagrelor 2. Aspirin	
Investigational Device	CytoSorb device	
Formulation, Dose, Route of Administration	1. Ticagrelor 90 mg twice daily, oral 2. Aspirin 75 mg once daily, oral	

iv. FUNDING AND SUPPORT IN KIND

FUNDERS	FINANCIAL AND NON FINANCIAL SUPPORT GIVEN
CytoSorbents	Funding of the study: trial set-up and running costs including staff costs, consumables, laboratory costs, MHRA fees and CytoSorb devices

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.
The funder, CytoSorbents Inc, will provide funding for all study activities.

vi. ROLES AND RESPONSIBILITIES OF TRIAL MANAGEMENT COMMITTEES/GROUPS

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

Given the small sample size and exploratory nature of the study, 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

Professor Rob Storey led the writing of this protocol and is CI for the study.

Dr Heather Judge assisted with designing laboratory methods.

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

viii. KEY WORDS:

Ticagrelor, aspirin, acute coronary syndromes, coronary artery bypass graft surgery, CytoSorb

ix. TRIAL FLOW CHART

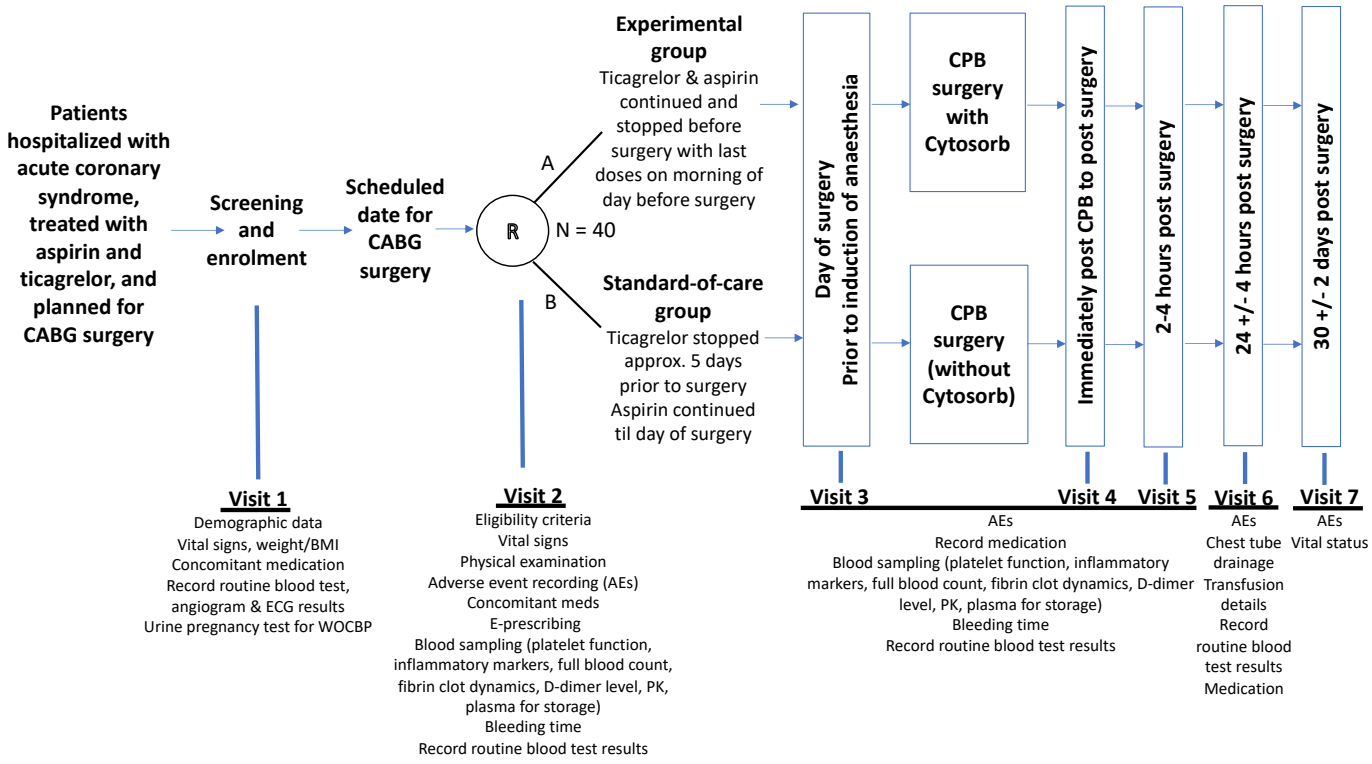

Figure 1A. Trial flow chart up to 12 May 2023 when 10 randomised patients had undergone CABG surgery. Abbreviations: AE: adverse events; BMI: body mass index; ECG: electrocardiogram; R: randomisation; CABG: coronary artery bypass graft; CPB: cardiopulmonary bypass; PK: pharmacokinetics; WOCBP: women of child-bearing potential.

Revised study design after 12 May 2023 for subsequent 30 patients:

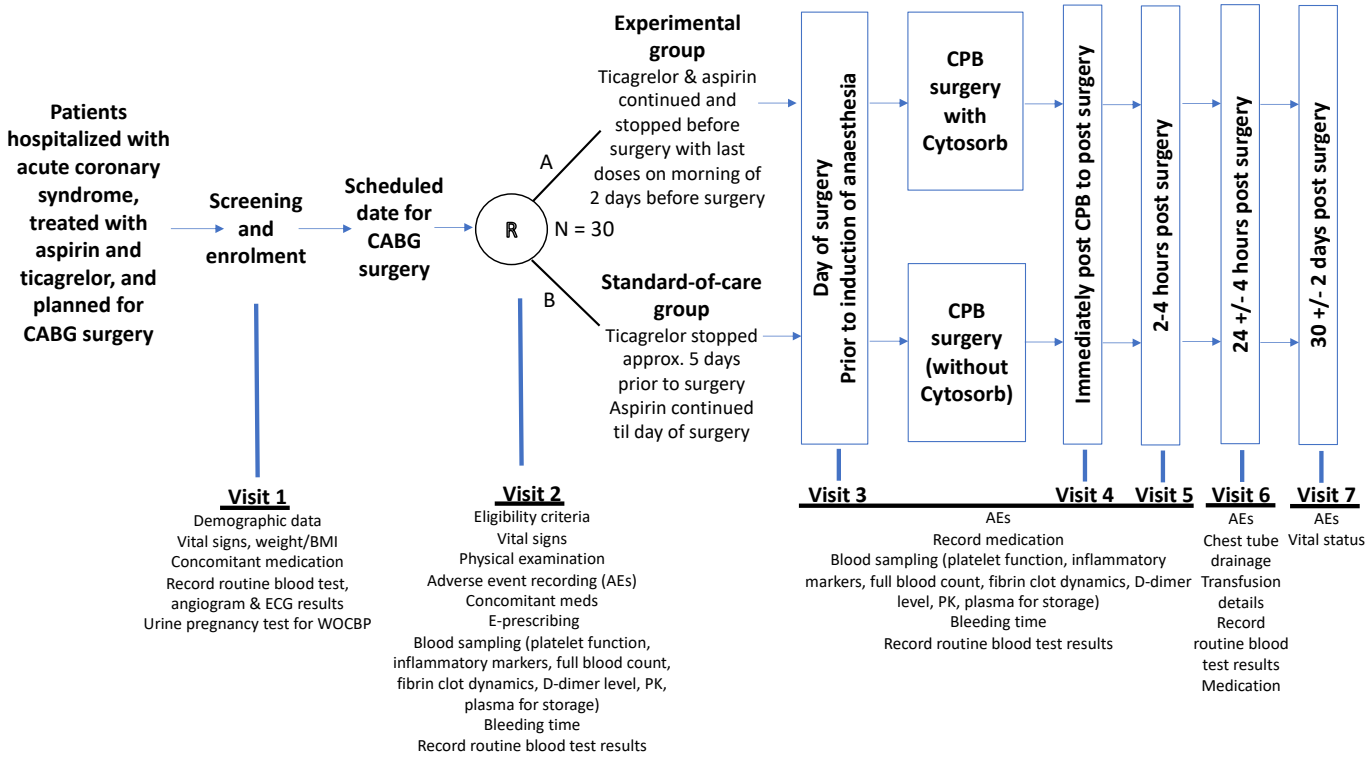

Figure 1B. Revised trial flow chart after 12 May 2023 for subsequent 30 patients. Abbreviations: AE: adverse events; BMI: body mass index; ECG: electrocardiogram; R: randomisation; CABG: coronary artery bypass graft; CPB: cardiopulmonary bypass; PK: pharmacokinetics; WOCBP: women of child-bearing potential.

1 BACKGROUND

Aspirin is an irreversibly-acting antiplatelet drug that works by inactivating platelet cyclo-oxygenase 1, thereby inhibiting platelet release of thromboxane A_2, a platelet-activator and vasoconstrictor. Ticagrelor is a reversibly-acting antiplatelet drug that reversibly binds to the platelet $P2Y_{12}$ receptor and prevents activation of this important amplification pathway by adenosine diphosphate (ADP). Both drugs are used as first-line treatment for patients presenting with acute coronary syndromes (ACS), the majority of which are caused by coronary artery thrombosis. Most ACS patients undergo coronary angiography in order to identify the extent of coronary artery disease and guide treatment, with the three principal options being percutaneous coronary intervention, coronary artery bypass graft (CABG) surgery, or no revascularisation procedure. Currently most patients with acute coronary syndromes in our centre are treated with aspirin and ticagrelor as first-line therapy prior to undergoing coronary angiography. Patients who have complex coronary artery disease on angiography may be referred for CABG surgery. The default strategy in these patients is to stop ticagrelor approximately 5 days before CABG surgery to prevent perioperative bleeding but this occasionally leads to recurrent myocardial infarction as the ticagrelor wears off, with additional risk if surgery is delayed. Some patients are treated in the days before CABG with intravenous tirofiban which is shorter acting but associated with increased bleeding risk and the optimum strategy for bridging antithrombotic therapy is unclear. A recent option that has emerged is the use of the CytoSorb device for removing ticagrelor from the blood during CABG surgery performed with cardiopulmonary bypass (CPB).[1, 2]

There are potential benefits of continuing ticagrelor and using the CytoSorb device in this setting beyond the obvious ones of preventing recurrent myocardial infarction (if ticagrelor is stopped 4-5 days before surgery) or bleeding (if tirofiban is used) before CABG. We (unpublished data on file) and others[3, 4] have shown that standard CABG surgery using cardiopulmonary bypass (CPB) leads to activation of clotting systems that can result in exhaustion of platelets and coagulation factors, consequently increasing bleeding risk. We have shown in other models how ticagrelor can reduce these effects[5] so having some ticagrelor present for at least part of the CPB period will likely reduce platelet and coagulation activation. CPB also leads to an inflammatory response that can cause lung injury[6] and we have similarly shown in other models how ticagrelor can reduce inflammatory response.[5] Furthermore, the CytoSorb device also removes cytokines from the circulation and is approved for this purpose.[7]
Aspirin is associated with diurnal variation in platelet inhibition related to peak-trough effect following each daily dose, with peak effects having significant impact on haemostasis compared to trough levels of platelet inhibition during maintenance therapy.[8] Consequently missing one or two daily doses of aspirin is expected to incrementally enhance collagen-induced platelet aggregation and improve haemostasis, despite the irreversible nature of aspirin's effects since new uninhibited platelets are continuously released into the circulation and each missed dose will lead progressively to more circulating uninhibited platelets. Missing a dose of aspirin the day before and on the day of CABG surgery is justifiable in patients who have also received ticagrelor but may constitute some risk if patients no longer have any platelet inhibition by ticagrelor and rely entirely on the protective effects of aspirin to prevent acute post-operative graft occlusion.

2 RATIONALE

Patients with ACS who are planned for CABG surgery have a high risk of recurrent myocardial infarction, cardiovascular death and bleeding related to the current standard management of antithrombotic therapy, which involves stopping ticagrelor 5 or more days prior to surgery and, for some patients, using intravenous tirofiban in the days prior to surgery. Activation of platelets and the coagulation system during CABG surgery can cause platelet dysfunction and coagulopathy that can lead to serious bleeding and requirement for transfusion of blood products, which are associated with increased morbidity. CABG surgery also causes an acute inflammatory response that may also increase morbidity and intensive care stays due to lung injury. Timing of CABG surgery is complicated by the routine practice of stopping ticagrelor 5 or more days prior since this can sometimes delay surgery or, because of scheduling uncertainties due to fluctuating availability of intensive care beds, lead to ticagrelor being stopped for longer periods than intended, thus increasing risk. Using an experimental strategy of continuing ticagrelor and aspirin until either one day (initial study design) or two days (study design after 12 May 2023) before CABG surgery and then using the CytoSorb system during CABG surgery to remove ticagrelor and cytokines may have the following benefits: (1) reduced pre-operative risk of recurrent myocardial infarction and death and/or reduced risk of bleeding associated with intravenous tirofiban; (2) reduced platelet and coagulation system activation during CABG surgery, thus offsetting the inhibitory effect of any residual ticagrelor at the end of CABG surgery; (3) reduced inflammatory response to CABG surgery; and (4) more efficient scheduling of CABG surgery with the potential to reduce pre-operative waits and overall length of hospitalisation.

2.1 Assessment and management of risk

Aspirin and ticagrelor are both widely used as antithrombotic drugs for the treatment of cardiovascular (CV) disease. The standard practice of stopping ticagrelor 5 days before CABG surgery is associated with risk of recurrent myocardial infarction and cardiovascular death whilst the selective practice, for some patients, of bridging with intravenous tirofiban before surgery is associated with increased risk of major bleeding. The experimental strategy of continuing ticagrelor until the day before CABG surgery and using the CytoSorb system to remove ticagrelor during surgery might increase the risk of bleeding if there is residual platelet inhibition from remaining ticagrelor at the end of surgery. However, it is expected that this will be counteracted by ticagrelor protecting against platelet activation and desensitisation during CABG surgery as well as by omitting a dose of aspirin on the day of surgery. The CytoSorb device has been extensively used in patients undergoing cardiac surgery and has been shown to be well tolerated and effective at removing potentially-harmful cytokines.[9] Following review of the first 10 study patients undergoing CABG surgery, the study was temporarily halted on 12 May 2023 due to concerns about bleeding in the experimental arm with three out of 5 patients in this arm undergoing reoperation for bleeding and one of these patients dying of multiorgan failure following two reoperations for bleeding compared with two out of 5 patients in the standard-of-care arm undergoing reoperation for bleeding with no deaths. These bleeding rates are higher than the approximately 10% reoperation rate usually observed in standard care. Inspection of the platelet function data in the experimental arm patients indicated that the CytoSorb device was not sufficiently reversing the effects of ticagrelor and missing a single dose of aspirin was not sufficient to compensate for the residual ticagrelor effects. This is consistent with data published online on 6 May 2023 showing that use of the Cytosorb device was associated with a 67% reduction in post-operative plasma ticagrelor levels in ACS patients undergoing CABG surgery, which may not be sufficient to impact on platelet inhibition at 24 hours after the last dose of ticagrelor. Observational data indicate that excess bleeding risk is avoided when ticagrelor has been stopped more than 72 hours before CABG surgery[10] and, given a half-life of ticagrelor of approximately 12 hours, it may be predicted that the impact of the CytoSorb device will be greatest when ticagrelor has been stopped 48 hours or more before CABG surgery. Furthermore, revising the study design so that aspirin is stopped two days rather than one day before CABG surgery is expected to allow twice as much recovery of platelet thromboxane A_2 generation due to twice as many circulating platelets that are uninhibited by aspirin, which will more effectively compensate for any effect of residual ticagrelor levels.

Known side-effect profiles:

Ticagrelor

Reported side effects of ticagrelor, reproduced from the Summary of Product Characteristics (SmPC) in use on 14 November 2021 [11], include:

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]		*Not known*: thrombotic thrombocytopenic purpura
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

Aspirin

Reported side effects of aspirin, reproduced from the SmPC in use on 14th November 2021 [12], include:

Blood and lymphatic system disorders	*Common:* Increased bleeding tendencies. *Rare:* Thrombocytopenia, granulocytosis, aplastic anaemia. *Not known:* Cases of bleeding with prolonged bleeding time such as epistaxis, gingival bleeding. Symptoms may persist for a period of 4–8 days after acetylsalicylic acid discontinuation. As a result, there may be an increased risk of bleeding during surgical procedures. Existing (haematemesis, melaena) or occult gastrointestinal bleeding, which may lead to iron deficiency anaemia (more common at higher doses).

Immune system disorders	*Rare:* Hypersensitivity reactions, angio-oedema, allergic oedema, anaphylactic reactions including shock.
Metabolism and digestive system disorders	*Not known:* Hyperuricemia, hypoglycaemia.
Nervous system disorders	*Rare:* Intracranial haemorrhage *Not known:* Headache, vertigo.
Ear and labyrinth disorders	*Not known:* Reduced hearing ability; tinnitus.
Vascular disorders	*Rare:* Haemorrhagic vasculitis.
Respiratory, thoracic and mediastinal disorders	*Uncommon:* Rhinitis, dyspnoea. *Rare:* Bronchospasm, asthma attacks.
Gastrointestinal disorders	*Common:* Dyspepsia, nausea, vomiting, diarrhoea. *Rare:* Severe gastrointestinal haemorrhage. *Not known:* Gastric or duodenal ulcers and perforation.
Hepatobiliary disorders	*Rare:* Reye's syndrome *Not known:* Hepatic insufficiency, hepatic enzyme increased
Skin and subcutaneous tissue disorders	*Uncommon:* Urticaria. *Rare:* Steven-Johnson syndrome, Lyells syndrome, purpura, erythema nodosum, erythema multiforme.
Renal and urinary tract disorders	*Not known:* Impaired renal function, salt and water retention.
Reproductive system and mammary disorders	*Rare:* Menorrhagia

Effects are graded as very common (≥1/10), common (≥1/100 to <1/10), uncommon (≥1/1,000 to <1/100), rare (≥1/10,000 to <1/1,000), very rare (<1/10,000) or not known (cannot be estimated from the available data).

CytoSorb device

The CytoSorb® 300 mL device is a sorbent-filled haemoperfusion cartridge (Figure 2) [13]. The cartridge consists of a cylinder and end-cap assembly filled with biocompatible porous polymer beads. At either end of the cylinder, a fine mesh screen is placed to retain the polymer beads within the device. Each end-cap has a standard blood tubing connector, which is compatible with standard CPB blood tubing lines.

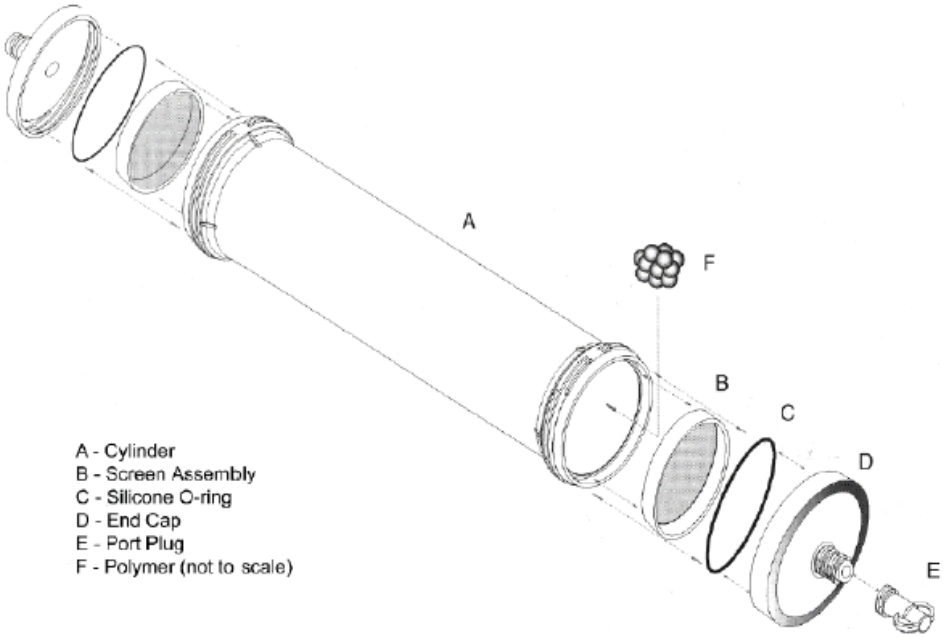

Figure 2. Expanded details of the CytoSorb 300 device

The polymer beads are composed of a divinylbenzene/polyvinyl pyrrolidone co-polymer, where each bead has hundreds of thousands of tightly controlled pores and channels that are generated via suspension polymerization. These pores and channels, in turn, enable the porous polymer beads to remove middle molecular weight substances between 5-kDa to 60-kDa, based on pore capture (size) and surface adsorption.

The polymer in the CytoSorb 300 mL device is also effective at binding small molecules with molecular moieties contained in ticagrelor. Ticagrelor in whole blood easily passes into the pores of the polymer where it adsorbs onto the internal polymer surface. This surface adsorption is governed by the hydrophobic nature of the polymer, through a combination of non-polar interactions, hydrogen bonding, and Van der Waals forces. These drug-polymer interactions favour removal of hydrophobic molecules over hydrophilic ones.

The CytoSorb 300 mL device is designed for use in extracorporeal circuits. For this investigation, the device will be incorporated as a component in a shunt of a standard CPB circuit and used to remove ticagrelor (Figure 3). The recommended blood flow rate through the device is 150-700mL/min with a target flow rate of 450 to 550 mL/minute (approximately 15% to 20% of a typical flow rate in CPB). The minimum and maximum

blood flow rates allowed through the device according to the Instructions for Use (IFU) are 100 mL/min and 700 mL/min, respectively.

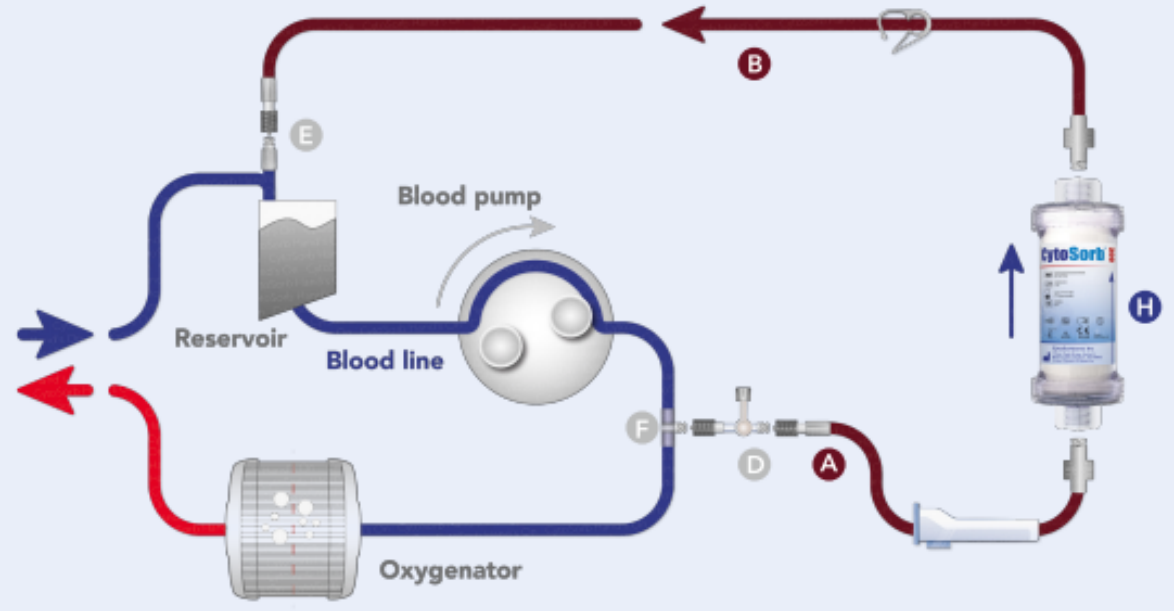

Figure 3. Set-up of the single-use CytoSorb device for removal of plasma ticagrelor and ticagrelor active metabolite as well as cytokines during cardiopulmonary bypass. CytoSorb (H) is mounted vertically using the mounting device. The device is incorporated into the cardiopulmonary bypass circuit via a shunt, utilizing a 3-way-valve to a Luer-Lock on the main line after the blood pump (F and D). Blood flows from the device back to the venous reservoir (B) and is connected via a high-flow Luer-Lock connection (E). If necessary, the volume of blood flow through device from the bypass circuit may be regulated by use of the roller clamp (A).

CytoSorb is CE-marked, meets the biocompatibility requirements of ISO 10993, and is approved for the removal of ticagrelor and cytokines, as well as bilirubin, myoglobin and rivaroxaban[13]. To date, more than 143,000 individual applications have been performed in more than 800 clinical settings worldwide, and the application has proven to be well-tolerated and safe[13]. The setup and application procedures are reported to be simple, safe and easy to perform[13]. The most important technical data (specifications) for the CytoSorb 300 adsorber are as follows[13]:

Details	dosage
CE Certified / notified body / QM-System	CE 0344 – DEKRA / ISO 13485:
Intended use:	CytoSorb is indicated for use in conditions where elevated levels of cytokines and/or bilirubin and/or myoglobin exist. CytoSorb is indicated for use intraoperatively during cardio-pulmonary bypass surgery for the removal of $P2Y_{12}$-Inhibitor ticagrelor and/or factor Xa-inhibitor rivaroxaban. CytoSorb may be used with extracorporeal blood circuit, such as intermittent haemodialysis, continuous renal replacement therapy (CRRT), cardiopulmonary bypass (CPB) and extracorporeal membrane oxygenation (ECMO)
Adsorber blood priming volume:	150 ml

Details	dosage
Blood flow rates min-max: Recommended blood flow rates:	100 – 700 ml/min 150 – 700 ml/min
Max. treatment time per device:	24 hours
Further details:	Latex- and PHT free product
Sterilization / shelf life / storage conditions:	Gamma sterilization / 3 years / 1°C to 40°C
Maximum pressure limit:	760 mmHg
Flow resistance (Hct 32 +/- 3% @ 37+/- 1°C):	Qb <= 700 ml/min 140 mmHg Qb <= 500 ml/min 090 mmHg Qb <= 200 ml/min 030 mmHg
GMDN Code:	34422
Surface:	Approximately 45,000 square meters
Adsorbent:	Proprietary and patented cross-linked divinylbenzene polymer, exclusively produced by CytoSorbents in the USA
Adsorption Spectrum:	Small and mid-size hydrophobic molecules up to a size of approximately 60kDa
Biocompatibility:	Biocompatibility tested as required in ISO10993
Mode of operation covered by IFU:	Many extracorporeal blood circuit (Hemoperfusion (HP), Intermittent hemodialysis (HD), continuous renal replacement therapy (CRRT), cardiopulmonary bypass (CPB), extracorporeal membrane oxygenation (ECMO/ECLS)
Priming fluid, procedure and duration:	Flushing with 2 litres of sterile isotonic saline (NaCL 0,9%) / Priming takes approximately 5 minutes. CytoSorb contains pre-loaded physiological saline with an approximate pH level of 6.8 pre-flush. CytoSorb does not require priming/coating with heparin
Therapy duration:	A single CytoSorb device can be used for up to 24hrs. Consider changing CytoSorb after 12hr for the first 24hrs when indicated, in cases of haemodynamic instability, high bilirubin, or no further substance removal.
Anticoagulation:	Possible with heparin or citrate

According to the current state of knowledge, there is no undesired elimination of physiological blood constituents, such as albumin and platelets, beyond that which can be expected within the scope of general haemofiltration or dialysis [13].

Management of medications in patients treated with CytoSorb during cardiopulmonary bypass

In some instances, concomitant medications administered during CPB can potentially be removed by the CytoSorb device. In the case of anaesthetic agents or vasopressors, these drugs are titrated to clinical effect as part of routine care and dosing should continue to be adjusted in accordance with observed clinical response.

Heparin is not removed by the CytoSorb device and can be dosed as per standard practice.

Some antimicrobial agents may be removed by CytoSorb therapy [14, 15](unpublished data provided by Cytosorbents). A number of these have been studied for removal in both benchtop and animal studies and are summarized as follows:

Low predicted removal (<30%) based on currently available data (*denotes drugs for which *in vivo* data is available)	**Moderate to high predicted removal (≥30%) based on currently available data (*denotes drugs for which *in vivo* data is available)**	**High removal (>60%) based on *in vitro* studies, but with large volume of distribution**
Categorizations listed here are based on existing *in vivo* studies (human data prioritized over animal) where available. In the absence of *in vivo* data, the categorization is based on available *in vitro* data. Guidance provided in this document is based on most current available information to date but is not exhaustive.		
Unlikely to be removed to a clinically significant extent with CytoSorb	CytoSorb may result in clinically meaningful removal and supplemental dosing should be considered	High *in vitro* removal does not necessarily translate to high removal *in vivo* due to the large volume of distribution. CytoSorb may not result in clinically meaningful removal.
amikacin anidulafungin* cefepime* ceftriaxone ciprofloxacin* clarithromycin* clindamycin* flucloxacillin ganciclovir* metronidazole* tobramycin imipenem netilmicin piperacillin*	linezolid* posaconazole* teicoplanin* meropenem* gentamicin amphotericin B liposomal* vancomycin fluconazole*	carbamazepine cyclosporine *remdesivir* voriconazole

Based on these studies, it is recommended that administration of antimicrobials for procedural prophylaxis be performed at least an hour before initiation of CytoSorb therapy to ensure adequate tissue distribution. Any impact of the CytoSorb device on removal of concomitant medications is influenced by duration of device exposure and is expected to be minor for CPB times of <90min. If the dose cannot be administered at least an hour before initiation of CytoSorb therapy and CPB is prolonged >90 mins, then consideration should be given to administering an additional dose (e.g. 30% of original dose) after completion of CPB surgery for the following drugs:

- Gentamicin
- Vancomycin
- Linezolid
- Meropenem

- Fluconazole
- Posaconazole
- Teicoplanin
- Liposomal amphotericin B

Mitigation of risks

Any risks in this study are mitigated by:

- a robust informed consent procedure, followed before any study-related activities take place
- ensuring at screening that potential participants do not have any ongoing bleeding or other tolerability concerns that would preclude safe involvement in the study with continued administration of either aspirin or ticagrelor
- excluding those with co-morbidities or receiving concomitant medication likely to significantly increase the risk of taking part in the study specifically with respect to ongoing exposure to aspirin or ticagrelor
- rigorous safety monitoring by the study team and Sponsor: this has led to modification of the protocol after 12 May 2023 and extension of the cessation time for ticagrelor and aspirin in the experimental arm from approximately 24 to 48 hours before surgery in light of review of bleeding outcomes and pharmacodynamic data
- an experienced multi-disciplinary team (cardiologist, cardiac surgeon, perfusionist, research coordinators, and research fellows) with ample past experience of conducting similar studies
- use of the CytoSorb device by a clinical care team that is experienced in its set-up and use for the removal of ticagrelor during CPB surgery
- a track record of safely performing similar studies of antiplatelet medication in patients with acute coronary syndromes

3 OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

3.1 Primary objective

The primary objective of this study is to compare the effects of the two treatment strategies on platelet aggregation responses to collagen 4 ug/mL immediately post CPB. We hypothesise that post-operative collagen-induced platelet aggregation responses will be similar in both groups.

3.2 Secondary objectives

The secondary objectives of this study are to compare the effects of the two treatment strategies on:

1. Platelet aggregation responses to arachidonic acid (AA) immediately post-CPB assessed using LTA.
2. Platelet aggregation response to ADP immediately post-CPB assessed using VerifyNow $P2Y_{12}$ assay.
3. Increase in plasma IL-6 level from pre-surgery to immediately post-CPB
4. Increase in plasma D-dimer level from pre-surgery to immediately post-CPB
5. Haemostasis (bleeding time) immediately post-surgery

6. Post-operative bleeding (chest tube drainage over 24 hours).

Further secondary objectives are to assess in each group separately:

1. Change in plasma ticagrelor levels from pre-surgery to immediately post-CPB
2. Change in plasma ticagrelor active metabolite levels from pre-surgery to immediately post-CPB

3.3 Tertiary objectives

The tertiary objectives of this study are to compare the effects of the two treatment strategies on:

1. Platelet aggregation responses to collagen 16 ug/mL, the combination of 5-hydroxytrypamine (5-HT) 1 umol/L with epinephrine 10 umol/L, and thrombin-receptor-activating peptide (TRAP) 8 and 32 umol/L pre-surgery and immediately post-CPB assessed using LTA.
2. Platelet aggregation response to ADP at pre-surgery assessed using VerifyNow $P2Y_{12}$ assay.
3. Platelet aggregation response to ADP at pre-surgery and immediately post-CPB assessed using Multiplate ADPtest assay.
4. Platelet aggregation response to TRAP pre-surgery and immediately post-CPB assessed using Multiplate TRAPtest assay.
5. Platelet $P2Y_{12}$-mediated response to ADP pre-surgery and immediately post-CPB assessed using VASP phosphorylation assay.
6. Serum thromboxane B_2 pre-surgery and immediately post-CPB
7. Increases in other inflammatory markers (plasma TNF-α; whole blood leukocyte count and differential; neutrophil and monocyte CD11b expression) from pre-surgery to immediately post-CPB
8. Unstimulated and ADP-stimulated platelet P-selectin expression pre-surgery and immediately post-CPB
9. Unstimulated and ADP-stimulated platelet-leukocyte aggregates pre-surgery and immediately post-CPB
10. Fibrin clot dynamics (lag time, lysis time, maximum turbidity) pre-surgery and immediately post-CPB
11. Bleeding time pre-surgery and change in bleeding time from pre-surgery to immediately post-surgery
12. Other measures of peri-operative bleeding (change in haematocrit from pre-surgery to immediately post-CPB, blood product transfusion, reoperation for bleeding).
13. Total length of hospital stay, time from coronary angiography to CABG surgery, time from randomisation to CABG surgery, length of stay from randomisation to hospital discharge, length of stay on intensive care unit.
14. Treatment-emergent adverse events occurring on or after first dose of study drug.

3.4 Outcome measures/endpoints

Primary endpoint/outcome

The primary endpoint will be the absolute level of platelet aggregation response to collagen 4 ug/mL immediately following CPB, assessed using LTA. Responses will be compared between the experimental and standard-of-care groups.

Secondary endpoints/outcomes

The two groups will be compared using the following outcomes, where 'pre-surgery' indicates the timepoint on the day of surgery prior to induction of anaesthesia:

1. Absolute LTA response to AA 1 mmol/L immediately post-CPB
2. Platelet reactivity units (PRU) and % inhibition values assessed using the VerifyNow $P2Y_{12}$ assay immediately post-CPB.

3. Increase in plasma IL-6 level, assessed using ELISA, from pre-surgery to immediately post-CPB
4. Increase in plasma D-dimer level, assessed using immunoturbidimetry, from pre-surgery to immediately post-CPB
5. Absolute skin bleeding time (in minutes) immediately post-surgery
6. Total chest tube drainage from immediately post-surgery to 24 hours post-surgery

The following outcomes will be assessed independently in each group:

1. Change in plasma ticagrelor concentration from pre-surgery to immediately post-CPB
2. Change in plasma ticagrelor active metabolite concentration from pre-surgery to immediately post-CPB

Tertiary endpoints/outcomes

The two groups will be compared using the following outcomes, where 'pre-surgery' indicates the timepoint on the day of surgery prior to induction of anaesthesia:

1. Absolute LTA response to collagen 4 ug/mL pre-surgery
2. Change in LTA response to collagen 4 ug/mL from pre-surgery to immediately post-CPB
3. Absolute LTA response to AA 1 mmol/L pre-surgery
4. Change in LTA response to AA 1 mmol/L from pre-surgery to immediately post-CPB
5. Absolute LTA responses to collagen 16 ug/mL, ADP 5 and 20 umol/L, 5-HT 1 umol/L + epinephrine 10 umol/L combination, and thrombin-receptor-activating peptide (TRAP) 8 and 32 umol/L pre-surgery
6. Absolute LTA responses to collagen 16 ug/mL, ADP 5 and 20 umol/L, 5-HT 1 umol/L + epinephrine 10 umol/L combination, and thrombin-receptor-activating peptide (TRAP) 8 and 32 umol/L immediately post-CPB
7. Change in LTA responses to collagen 16 ug/mL, ADP 5 and 20 umol/L, 5-HT 1 umol/L + epinephrine 10 umol/L combination, and thrombin-receptor-activating peptide (TRAP) 8 and 32 umol/L from pre-surgery to immediately post-CPB
8. Absolute VerifyNow PRU values pre-surgery
9. Change in VerifyNow PRU values from pre-surgery to immediately post-CPB
10. Absolute VerifyNow % inhibition values pre-surgery
11. Absolute VerifyNow % inhibition values immediately post-CPB
12. Change in VerifyNow % inhibition values from pre-surgery to immediately post-CPB
13. Absolute Multiplate ADPtest AUC values pre-surgery (AUC being the assay-specific unit determined from the area-under-the-curve of the aggregation response)
14. Absolute Multiplate ADPtest AUC values immediately post-CPB
15. Change in Multiplate ADPtest AUC values from pre-surgery to immediately post-CPB
16. Absolute Multiplate TRAPtest AUC values pre-surgery
17. Absolute Multiplate TRAPtest AUC values immediately post-CPB
18. Change in Multiplate TRAPtest AUC values from pre-surgery to immediately post-CPB
19. Absolute VASP PRI values pre-surgery
20. Absolute VASP PRI values immediately post-CPB
21. Change in VASP PRI values from pre-surgery to immediately post-CPB
22. Absolute serum thromboxane B_2 levels, assessed using ELISA, pre-surgery
23. Absolute serum thromboxane B_2 levels, assessed using ELISA, immediately post-CPB
24. Change in serum thromboxane B_2 levels, assessed using ELISA, from pre-surgery to immediately post-CPB
25. Absolute unstimulated platelet P-selectin expression immediately post-CPB
26. Change in unstimulated platelet P-selectin expression from pre-surgery to immediately post-CPB
27. Absolute platelet P-selectin expression induced by ADP 0.3-30 umol/L pre-surgery
28. Absolute platelet P-selectin expression induced by ADP 0.3-30 umol/L immediately post-CPB

29. Change in platelet P-selectin expression induced by ADP 0.3-30 umol/L from pre-surgery to immediately post-CPB
30. Increase in plasma TNF-α level, assessed using ELISA, from pre-surgery to immediately post-CPB
31. Increase in unstimulated neutrophil CD11b expression, assessed using flow cytometry, from pre-surgery to immediately post-CPB
32. Increase in unstimulated monocyte CD11b expression from pre-surgery to immediately post-CPB
33. Increase in unstimulated platelet-monocyte aggregates from pre-surgery to immediately post-CPB
34. Increase in unstimulated platelet-neutrophil aggregates from pre-surgery to immediately post-CPB
35. Absolute platelet-monocyte aggregates stimulated by ADP 30 umol/L pre-surgery
36. Absolute platelet-monocyte aggregates stimulated by ADP 30 umol/L immediately post-CPB
37. Absolute platelet-neutrophil aggregates stimulated by ADP 30 umol/L pre-surgery
38. Absolute platelet-neutrophil aggregates stimulated by ADP 30 umol/L immediately post-CPB
39. Change in fibrin lag time from pre-surgery to immediately post-CPB
40. Change in fibrin clot lysis time from pre-surgery to immediately post-CPB
41. Change in fibrin clot maximum turbidity from pre-surgery to immediately post-CPB
42. Absolute skin bleeding time pre-surgery
43. Change in skin bleeding time from pre-surgery to immediately post-surgery
44. Absolute plasma ticagrelor concentration pre-surgery
45. Absolute plasma ticagrelor concentration immediately post-CPB
46. Absolute plasma ticagrelor active metabolite concentration pre-surgery
47. Absolute plasma ticagrelor active metabolite concentration immediately post-CPB
48. Change in whole blood platelet count from pre-surgery to immediately post-CPB
49. Change in haematocrit from pre-surgery to immediately post-CPB
50. Change in whole blood total leukocyte and differential counts from pre-surgery to immediately post-CPB
51. Total number of units of blood products and type transfused from pre-surgery to 24 hours post-surgery.
52. Total length in days of hospital stay, time in days from coronary angiography to CABG surgery, time in days from randomisation to CABG surgery, length of stay in days from randomisation to hospital discharge, length of stay in days on intensive care unit.

In addition, measurements taken at randomisation, the changes from randomisation to immediately pre-surgery, the measurements taken at 2 hours post-surgery and the changes from either pre-surgery or immediately post-CPB to 2 hours post-surgery will be presented descriptively. The number of patients with thrombotic and/or bleeding events and other treatment-emergent adverse events occurring between randomisation and CABG surgery as well as the number of patients requiring reoperation for bleeding after CABG surgery will also be presented descriptively.

4 TRIAL DESIGN

The trial is a pharmacodynamic study to determine the effects on post-operative platelet aggregation responses of a strategy of continuing ticagrelor and aspirin until either the day before CABG surgery (first 10 randomised patients undergoing surgery prior to 12 May 2023) or two days before CABG surgery (subsequent 30 randomised patients undergoing surgery) then using the CytoSorb device during surgery compared with a routine strategy of stopping ticagrelor approximately 5 days before CABG surgery (unless platelet function testing at 3 or 4 days indicates recovery of platelet reactivity; see Appendix A for local guideline) and continuing aspirin up until the time of surgery.

We will perform an open-label, single-centre, randomised clinical trial of two independent parallel groups of participants with acute coronary syndromes who are currently treated with aspirin 75mg once daily and ticagrelor 90mg twice daily and are planned for urgent CABG surgery (total n=40). Those who are receiving oral antithrombotic therapy apart from aspirin and ticagrelor will be excluded. The initial study design was that participants will be randomised to either discontinue ticagrelor and aspirin on the day prior to CABG surgery (last doses in the morning of the day before surgery) or to discontinue ticagrelor according to standard practice approximately 5 days before CABG surgery and continue aspirin until the time of surgery. After review of data on the first 10 randomised patients undergoing CABG surgery, the trial was halted on 12 May 2023 in order to change the protocol so that the experimental group discontinued ticagrelor and aspirin two days prior to CABG surgery (last doses in the morning of two days before surgery). Patients randomised to the first group will receive CytoSorb therapy during cardiopulmonary bypass in order to reduce the plasma levels of ticagrelor and its active metabolite. Randomisation will be in a 1:1 fashion using SealedEnvelope, a commercially available service for this purpose. Patients will receive standard-of-care antiplatelet therapy following CABG surgery, usually consisting of further dosing of aspirin following surgery when haemostasis is secured and restarting ticagrelor the day following surgery. Established markers of platelet function (light transmittance aggregometry, P-selectin expression, VerifyNow $P2Y_{12}$ assay, Multiplate ADPtest and TRAPtest, VASP phosphorylation assay, serum thromboxane B_2), inflammation (plasma IL-6, TNF-α, leukocyte count and subset, neutrophil CD11b expression, platelet-monocyte aggregates), and coagulation (fibrin clot dynamics, fibrinolysis and plasma D-dimer), as well as plasma levels of ticagrelor and its active metabolite will be measured at the time of randomisation, immediately before induction of anaesthesia for CABG surgery, immediately after completion of CPB and 2 hours after surgery. Haemostasis (bleeding time) will be assessed at the time of randomisation, immediately before induction of anaesthesia for CABG surgery, immediately after surgery and 2-4 hours after surgery. In addition, we will store plasma samples at all time points for subsequent analysis of biomarkers of relevance to CV disease.

5 TRIAL SETTING

Visits will be carried out in the Cardiology and Cardiothoracic Surgery Directorate at the Northern General Hospital (NGH). Laboratory analyses will be conducted in the Cardiovascular Research Unit (within the Clinical Research Facility, Northern General Hospital). Safety blood tests will be performed as part of standard care within the Sheffield Teaching Hospital (STH) laboratories.

Participants will be patients with a diagnosis of acute coronary syndromes who have undergone invasive coronary angiography and are planned for urgent CABG surgery during the same admission. There is anticipated to be an adequate population of eligible potential participants under the care of the Cardiology and Cardiothoracic Surgery Directorate of Sheffield Teaching Hospitals NHS Foundation Trust, meaning a single-centre design is appropriate without the need for any separate participant identification centres.

6 PARTICIPANT ELIGIBILITY CRITERIA

To participate in this trial, subjects must meet all of the following inclusion criteria and none of the exclusion criteria:

6.1 Inclusion criteria

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

2. Male or female aged 18 years or greater

3. Currently hospitalised for treatment of an acute coronary syndrome

4. Currently receiving aspirin 75mg once daily

5. Current treatment or prior treatment during the current hospitalisation with ticagrelor 90mg twice daily

6. Currently being considered for CABG surgery during the current hospitalisation following coronary angiography with plan to stop ticagrelor approximately 5 days before surgery

6.2 Exclusion criteria

1. CABG surgery planned to occur urgently either less than 3 days after cessation of ticagrelor or less than 5 days after cessation of ticagrelor without guidance by platelet function testing

2. Treatment within the last 7 days or planned treatment with any antiplatelet drug other than aspirin and ticagrelor (including prasugrel, clopidogrel, dipyridamole, cilostazol, or glycoprotein IIb/IIIa antagonists) except for a short course of a glycoprotein IIb/IIIa antagonist (tirofiban or eptifibatide) as bridging therapy in those discontinuing ticagrelor at least 3 days prior to scheduled CABG surgery

3. Current or planned treatment prior to CABG surgery with oral or parenteral anti-inflammatory/immunomodulatory drugs (oral corticosteroids; disease-modifying anti-rheumatic drugs, including methotrexate at any dose; immunosuppressants; chemotherapy drugs), oral anti-coagulant medications (warfarin, dabigatran, rivaroxaban, edoxaban, apixaban) or intravenous fibrinolytic agents

4. Current or planned treatment prior to CABG surgery with a non-steroidal anti-inflammatory drug other than aspirin

5. Known hypersensitivity to or intolerance of aspirin, salicylic acid (including certain asthma patients who may suffer an asthma attack or faint), ticagrelor or excipients

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

7. Abnormal full blood count on blood tests performed during the current hospitalisation that, in the opinion of the investigator, would preclude safe involvement in the study or compromise its scientific credibility

8. Evidence of active pathological bleeding

9. Participants with clinically significant co-morbidity that, in the opinion of the investigator, would preclude safe involvement in the study or compromise its scientific credibility

10. Any clinically significant abnormal laboratory test results during the current hospitalisation that, in the opinion of the investigator, would preclude safe involvement in the study
11. Pregnant or breast-feeding women
12. Known haemorrhagic diathesis or coagulation disorders such as haemophilia or moderate or severe thrombocytopenia (platelet count < 100 x 10^9/L)
13. Current treatment with a strong CYP3A inhibitor or inducer (see section 8.9)
14. Current treatment with doses of simvastatin or lovastatin >40mg/day or CYP3A substrates with a narrow therapeutic index (see section 8.9)
15. Any other contraindication for ticagrelor or aspirin treatment as detailed in the respective SmPCs
16. Women of child-bearing potential (WOCBP)[A] unless negative pregnancy test at screening and willing to use highly-effective contraception[B] for the duration of treatment with study medication

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

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

7 TRIAL PROCEDURES

7.1 Recruitment

7.1.1 Participant identification

Potential participants with ACS who are planned for urgent CABG surgery will be identified from the surgical waiting list and from the cardiac catheterisation laboratory following coronary angiography where an initial approach will be made by the study team clinicians who are also members of the Cardiology and Cardiothoracic Surgery directorate clinical care team (Prof Rob Storey, Mr Steven Hunter, Dr Nadir Elamin, Dr William Parker). In addition, other cardiology and cardiac surgery consultants within the directorate will be contacted and asked to approach potential participants to invite them, and share the details of potential participants who agree to be contacted by the study investigators. Potential participants who are interested in taking part in the study will then be provided with the patient information sheet and consent form.

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 including a full enquiry into CV symptoms and recording of concomitant medications
- Physical examination
- Collection of demographic data
- Recording of vital signs (pulse, blood pressure and temperature)
- Recording of weight and BMI
- Review and recording of results of standard-of-care blood tests, including full blood count, urea and electrolytes, liver function tests and coagulation tests.
- Recording of results of coronary angiogram and ECGs
- Review of medical records to confirm that the patient is being considered for CABG surgery during the current hospitalisation
- Urine pregnancy test for WOCBP

An anonymised log will be kept of screened patients (i.e. ACS patients who are planned for urgent CABG surgery) indicating date of screening, eligibility, whether or not eligible patients were enrolled, and, if appropriate, reasons for ineligibility or reasons why eligible patients were not included in the study.

7.1.3 Payment

Participants will not receive any reimbursement for their involvement in the study.

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 PIS/ICF 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 their further treatment and will be provided with a contact point where they 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 PIS/ICF.

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 plasma for future, as yet unplanned, studies. Explicit consent for this will be sought on the ICF at enrolment. Any cellular samples taken will be destroyed before the end of the study.

7.3 The randomisation scheme

Participants will be randomised to one of the following two strategies in a 1:1 fashion:

(A) Continue or restart ticagrelor 90mg twice daily and continue aspirin 75mg once daily until either the day before CABG surgery (first 10 randomised patients undergoing surgery prior to 12 May 2023) with the last doses in the morning on that day or two days before CABG surgery (modified study design for subsequent 30 randomised patients undergoing surgery) with the last doses in the morning on that day. The CytoSorb system will be used at the same time as cardiopulmonary bypass during surgery in order to reduce the plasma levels of ticagrelor and its active metabolite.

or

(B) Stop ticagrelor 5 or more days before scheduled CABG surgery and continue aspirin 75mg once daily, taking the last dose in the morning prior to surgery. CABG surgery may proceed at 3 or 4 days after last dose of ticagrelor if pre-operative platelet function testing indicates recovery of platelet reactivity, as per standard practice (see Appendix A for local clinical protocol).

7.3.1 Method of implementing the randomisation/allocation sequence

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

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

The system will generate an immediate email to the investigators stating the treatment allocation and this will be printed and placed in the participant's study file.

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 (screening)

- Medical history
- Demographic data
- Concomitant medications
- Vital signs (pulse, blood pressure and temperature)
- Weight and BMI
- Review and recording of results of standard-of-care blood tests, including full blood count, urea and electrolytes, liver function tests and clotting screen,
- Recording or results of coronary angiography and ECGs

- Urine pregnancy test for women of child-bearing potential

7.7 Trial assessments

Visit 1 - Screening (Day -21 to 0)

Screening of subjects and all study-related procedures will take place on the wards of Sheffield Teaching Hospital NHS Foundation Trust. The following assessments and procedures will be performed:

- Full informed consent, including completion of the ICF
- Inclusion/exclusion criteria (see section 6)
- Review of medical history
- Recording of concomitant medications
- Physical examination
- Recording of demographic data
- Recording of most recent vital signs (pulse, blood pressure and temperature) assessed as part of routine care
- Recording of weight and BMI
- Recording of results of coronary angiogram and ECGs
- Review of medical records to confirm that the patient is being considered for CABG surgery during the current hospitalisation
- Review of the results of routine blood tests performed during the current hospitalisation (full blood count, urea & electrolytes, liver function tests, coagulation tests)
- Urine pregnancy test for WOCBP

If eligible, the study team will monitor progress of the patient to ascertain when they are accepted for CABG surgery, at which stage they may proceed to randomisation. Subjects who fail screening will be recorded on a screen failure log with the reason for failure.

Visit 2 (Day 0) – Randomisation

- Reconfirm eligibility criteria met (by a medically qualified member of the study team, see section 6) and no withdrawal criteria met (section 7.10)
- Adverse event recording, including recording of any thrombotic or bleeding events occurring since Visit 1
- Record most recent vital signs: pulse, blood pressure and temperature
- Physical examination
- Recording of any changes to concomitant medications since Visit 1
- Randomisation and recording of planned treatment strategy in the medical records

- Electronic prescribing of ticagrelor for group A if this has been discontinued, and prescribing of the planned discontinuation time for ticagrelor and, for group A, aspirin
- 32 ml venous blood samples for baseline platelet function, inflammatory markers, full blood count including leukocyte differential, fibrin clot dynamics, plasma D-dimer level, plasma levels of ticagrelor and its active metabolite, and plasma for storage
- Bleeding time measurement
- Recording of the results of routine blood tests performed since Visit 1 (full blood count, urea & electrolytes, liver function tests, coagulation tests)

Medication period (variable according to timing of surgery and randomised group allocation)

- Participants randomised to the experimental group (group A) will continue ticagrelor 90mg twice daily and aspirin 75 mg once daily until either the day before scheduled surgery and will take the last pre-surgery doses of ticagrelor and aspirin in the morning on the day before surgery (first 10 randomised patients undergoing surgery before 12 May 2023) or two days before scheduled surgery and will take the last pre-surgery doses of ticagrelor and aspirin in the morning of two days before surgery. These participants will receive CytoSorb therapy during surgery.
- Participants randomised to the control group (group B) will take their last dose of ticagrelor 90mg approximately 5 days before scheduled surgery, as per routine care, and will continue taking aspirin 75mg once daily, including on the day of scheduled surgery.

The medication period may last from less than 1 day in group A patients who are scheduled to undergo surgery either the day following randomisation (first 10 randomised patients undergoing surgery prior to 12 May 2023) or two days following randomisation (subsequent 30 randomised patients undergoing surgery) to up to 21 days in patients where surgery is delayed because of intercurrent illness or problems with theatre or intensive care capacity, such as related to respiratory illness epidemic/pandemic.

Visit 3 (day of scheduled surgery, before induction of anaesthesia)

This visit will occur before and as close as possible to induction of anaesthesia but no more than 4 hours before induction of anaesthesia. The following will be performed:

- Reconfirm eligibility criteria met (by a medically qualified member of the study team, see section 6) and no withdrawal criteria met (section 7.10)
- Adverse event recording, including recording of any thrombotic or bleeding events occurring since Visit 2
- 32 ml venous blood samples for platelet function, inflammatory markers, full blood count including leukocyte differential, fibrin clot dynamics, plasma D-dimer level, plasma levels of ticagrelor and its active metabolite, full blood count including leukocyte differential, and plasma for storage
- Bleeding time measurement
- Recording of times and dates of last doses of ticagrelor and aspirin

- Recording of any changes in concomitant medication since Visit 2
- Recording of the results of routine blood tests performed since Visit 2 (full blood count, urea & electrolytes, liver function tests, coagulation tests)

In the event that surgery is delayed so that induction of anaesthesia will be more than 4 hours after the visit has occurred, the visit will be repeated and the samples and results from the previous visit 3 will be discarded.

Visit 4 (day of surgery, after completion of cardiopulmonary bypass to immediately after completion of surgery)

- 32 ml venous blood samples for platelet function, inflammatory markers, full blood count including leukocyte differential, fibrin clot dynamics, plasma D-dimer level, plasma levels of ticagrelor and its active metabolite, full blood count including leukocyte differential, and plasma for storage immediately post-CPB. When possible, perfusionist or anaesthetist members of the clinical team will draw these blood samples to pass to the research team in the operating theatre. The samples should be collected as soon as possible after completion of cardiopulmonary bypass but, where collection in the operating theatre is not feasible, may be collected as soon as possible and not more than 1 hour after completion of surgery (defined as transfer from the operating theatre to the recovery area).
- Bleeding time measurement immediately after completion of surgery

Visit 5 (day of surgery, 2-4 hours after completion of surgery)

- 32 ml venous blood samples for platelet function, inflammatory markers, full blood count including leukocyte differential, fibrin clot dynamics, plasma D-dimer level, plasma levels of ticagrelor and its active metabolite, full blood count including leukocyte differential, and plasma for storage
- Bleeding time measurement

Visit 6 (24 ± 4 hours after completion of surgery)

- Adverse event recording, including recording of any thrombotic or bleeding events, occurring since Visit 3
- Recording of chest tube drainage, blood product transfusions, and bleeding events, including procedures or reoperation for management of bleeding
- Recording of dose and time/date of any antiplatelet medication given post-surgery
- Recording of any changes in concomitant medication since Visit 3.
- Recording of the results of routine blood tests performed since Visit 3 (full blood count, urea & electrolytes, liver function tests, coagulation tests)

Visit 7 (30 ± 2 days after surgery, telephone follow-up)

- Vital status

- Recording of additional chest tube drainage, blood product transfusions, and bleeding events, including procedures or reoperation for management of bleeding, since Visit 6
- Recording of any changes in concomitant medication since Visit 6.
- Recording of the results of routine blood tests performed since Visit 6 (full blood count, urea & electrolytes, liver function tests, coagulation tests)
- Recording of date of transfer from intensive care unit to surgical ward
- Recording of date of discharge from hospital
- Adverse event recording, including recording of any thrombotic or bleeding events, occurring since Visit 6

General overview of blood sample analysis

The aim of the analysis of study samples will be to demonstrate the effects of the two strategies of cessation of ticagrelor and aspirin before CABG surgery on platelet function, inflammation, fibrin clot dynamics, fibrinolysis, and haemostasis in participants undergoing CABG surgery for the management of ACS.

Platelet activation and aggregation will be assessed by (1) light transmittance aggregometry using collagen 4 and 16 ug/mL, AA 1 mmol/L, ADP 5 and 20 umol/L, 5-HT 1 umol/L + epinephrine 10 umol/L combination, and thrombin-receptor-activating peptide (TRAP) 8 and 32 umol/L as agonists, (2) measurement of P-selectin expression stimulated by ADP 0.3, 1, 3, 10 and 30 umol/L and unstimulated, (3) VerifyNow $P2Y_{12}$ assay, (4) Multiplate ADPtest and TRAPtest assays, (5) VASP phosphorylation assay, and (6) measurement of serum thromboxane B_2 levels.

Effects on the inflammatory state and response to CABG surgery will be assessed by measurement of (1) plasma IL-6 level, (2) plasma TNF-α level, (3) whole blood leukocyte counts including enumeration (% and absolute numbers) of leukocyte subsets, (4) measurement of platelet-monocyte and platelet-neutrophil aggregates, unstimulated and stimulated by ADP 30 umol/L, (5) unstimulated neutrophil CD11b expression, and (6) unstimulated monocyte CD11b expression.

Parameters of fibrin clot formation and lysis, specifically lag time, lysis time and final clot turbidity, will be assessed using a validated turbidimetric plasma assay. *In vivo* thrombus formation will be assessed by measuring plasma D-dimer level.

Haemostasis will be assessed by measuring bleeding time at baseline, pre-surgery, immediately post-surgery and 2 hours post-surgery using a method shown to be sensitive to additive effects of antiplatelet agents [16]. The procedure for measuring bleeding time is described in the study-specific SOP relating to this.

Study samples will either be measured immediately after collection (e.g., LTA, Multiplate, VerifyNow, flow cytometric assays) or stored for assays at a later time (other endpoints). We will also store acellular samples of plasma within the University of Sheffield

laboratories for future, as yet unplanned, studies for 2 years after study completion. Consent will be sought for this at study enrolment.

Study investigations will be performed at the following time points:

1. Platelet function tests
- LTA using collagen 4 and 16 ug/mL, AA 1 mmol/L, ADP 5 and 20 umol/L, 5-HT 1 umol/L + epinephrine 10 umol/L combination, and thrombin-receptor-activating peptide (TRAP) 8 and 32 umol/L as agonists: visits 2, 3, 4 and 5.
- P-selectin expression stimulated by ADP 0.3, 1, 3, 10 and 30 umol/L and unstimulated: visits 2, 3, 4 and 5.
- Multiplate ADPtest and TRAPtest assays: visits 2, 3, 4 and 5.
- VerifyNow $P2Y_{12}$ assay: visits 2, 3, 4 and 5.
- VASP phosphorylation assay: visits 2, 3, 4 and 5.
- Serum thromboxane B_2 levels: visits 2, 3, 4 and 5.

2. Inflammatory markers
- Plasma IL-6: visits 2, 3, 4 and 5.
- Plasma TNF-α: visits 2, 3, 4 and 5.
- Circulating leukocyte count plus subsets (differential leukocyte count): visits 2, 3, 4 and 5.
- Measurement of platelet-monocyte and platelet-neutrophil aggregates: visits 2, 3, 4 and 5.
- Monocyte CD11b expression: visits 2, 3, 4 and 5.
- Neutrophil CD11b expression: visits 2, 3, 4 and 5.

3. Fibrin clot dynamics/regulation of fibrinolysis/*in vivo* thrombosis
- Lag time, lysis time, final clot turbidity: visits 2, 3, 4 and 5.
- Plasma D-dimer level: visits 2, 3, 4 and 5.

4. Ticagrelor pharmacokinetics
- Plasma levels of ticagrelor and ticagrelor active metabolite: visits 2, 3, 4 and 5.

5. Haemostasis
- Bleeding time: visits 2, 3, 4 and 5.

6. Samples for storage
- Plasma for storage: visits 2, 3, 4 and 5.

7.8 Long term follow-up assessments

At visit 7 (30 ± 2 days after CABG surgery) participants will be followed up via telephone. Any new adverse events will be recorded. Participants will be thanked for their involvement in the study. In the event of a participant remaining uncontactable at the end of the follow-up window, we will interrogate the hospital records of the participant to clarify vital status and any evidence of untoward events. Participants will be declared 'lost to follow up' if they are uncontactable 28 days after visit 7. A file note will be made in this instance.

7.9 Qualitative assessments

Not applicable to this study.

7.10 Withdrawal or postponement criteria

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

- Decision not to proceed with CABG surgery
- 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 this involves delaying CABG surgery by less than 21 days after randomisation and further study activities can be appropriately postponed until the subject has sufficiently recovered and appropriate adaptation of the dosing schedule of the study medication is feasible (see section 7.10.1).
- Development of an indication for non-study antithrombotic or other medication that, in the opinion of the investigator, is likely to affect thrombosis, inflammation or haemostasis at Visit 2, 3, 4 or 5.
- Non-adherence to the randomised dosing strategy for study medications during the 5 days preceding Visit 3 sufficient to affect the comparison of the two strategies e.g., aspirin dose given on the day of surgery in group A or ticagrelor given within 3 days of surgery in group B
- Administration of a glycoprotein IIb/IIIa antagonist less than 4 hours before visit 3
- 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 or discontinuation could cause potential risks to the subjects.

  In the event of meeting one or more of these criteria:

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

- Participants who receive at least one dose of IMP following randomisation will be followed up by telephone at 30 +/- 2 days after withdrawal, and additionally all who discontinue due to adverse events (AEs) will be followed up until resolution or stabilisation, with all outcomes of AEs recorded in the CRF.

- Participants who are withdrawn before undergoing CABG surgery will be replaced whereas participants who are withdrawn after undergoing CABG surgery will not be replaced.

7.10.1 Provision for postponement of planned CABG surgery

If CABG surgery does not proceed on the scheduled date, then study medication will be handled according to the randomised group:
(1) Participants randomised to group A will have their medication handled as follows:
Prior to 12 May 2023: either continue ticagrelor and aspirin, if still receiving these, until the morning of the day before the next scheduled date of surgery or, if ticagrelor and aspirin have been discontinued, restart ticagrelor with a stat dose of 90 mg and aspirin with a stat dose of 75mg, following which the date of discontinuation of ticagrelor and aspirin will be rescheduled according to the rescheduled date for CABG surgery.

Revised protocol dated 19 May 2023: if surgery is rescheduled for the following day then give a single loading dose of aspirin 300 mg in the morning of the postponement date and no further doses of ticagrelor or aspirin; if surgery is rescheduled for two days' time then give loading doses of ticagrelor 180mg and aspirin 300mg in the morning of the postponement date and no further doses of ticagrelor or aspirin; if surgery is rescheduled for more than two days' time then give single loading doses of ticagrelor 180mg and aspirin 300mg followed by ticagrelor 90mg bd and aspirin 75mg od with last doses of ticagrelor and aspirin given on the morning of two days before the scheduled date of CABG surgery.
(2) Participants randomised to group B who have discontinued ticagrelor will remain off ticagrelor if CABG surgery has been rescheduled for a date within the next 5 days and bridging antithrombotic therapy (e.g., tirofiban) may be commenced at the discretion of the clinical care team, according to routine clinical practice. If CABG surgery has been rescheduled for a date more than 5 days in the future, then ticagrelor may be restarted at the discretion of the clinical care team and then discontinued 5 days before the rescheduled date of CABG surgery.
If the patient does not proceed to CABG surgery either prior to hospital discharge or within 21 days after randomisation, they will be withdrawn from the study and will be replaced.

7.11 Storage and analysis of clinical samples

The samples will be stored and analysed according to the study-specific laboratory manual. Samples are anticipated to be analysed by the following laboratories and methods:
Laboratory analyses from primary, secondary and tertiary endpoints, except for pharmacokinetic analyses, will be conducted in the Cardiovascular Research Unit (within the Clinical Research Facility) and have been previously validated in clinical studies, as per the cited publications:

1. Platelet aggregation responses to collagen, arachidonic acid, ADP, 5-HT + epinephrine combination, and TRAP by light transmittance aggregometry [17-19]
2. P-selectin expression using flow cytometry [18]
3. Multiplate ADPtest and TRAPtest assays [20]
4. VerifyNow $P2Y_{12}$ assay [19]
5. VASP phosphorylation assay using flow cytometry [18]
6. Serum thromboxane B_2 levels by ELISA [17]
7. Plasma IL-6 by enzyme-linked immunosorbent assay (ELISA) [21]
8. Plasma TNF-α ELISA [21]
9. Leukocyte count and differential subsets by automated cell counting [21, 22]
10. Platelet-leukocyte aggregates phenotype and enumeration using multi-colour flow cytometry [21, 22]
11. Monocyte and neutrophil CD11b expression using multi-colour flow cytometry [21, 22]
12. Fibrin clot lysis time by fibrin clot turbidimetry [23]
13. Fibrin clot lag time by fibrin clot turbidimetry [23]
14. Fibrin clot final turbidity by fibrin clot turbidimetry [23]

Laboratory assessments of platelet and leukocyte function carried out in the Cardiovascular Research Unit Laboratory will adhere to an SOP that will provide comprehensive laboratory quality control. This SOP has been produced in accordance with Medicines for Human Use (Clinical Trials) Regulations 2004 and subsequent amendments and ICH Good Clinical Practice (GCP) & Research Governance Framework. This SOP will ensure the general processes required to guarantee quality standards are

maintained for aforementioned assays. The SOP imposes checks on: calibration and maintenance of equipment, daily quality control, reagent stability and batch variability, method validation, data recording and audit, exceptional results, staff training, sample receipt and data archiving.
Further study-specific quality control checks of assays that inform the primary and secondary endpoints will be performed on a monthly basis in the Cardiovascular Research Unit laboratory using the quality control systems as detailed below:

1. Light transmittance aggregometry – LTA-Check. This is a lypophilized preparation of platelets that will be used to check that the output from the aggregometer is within the prescribed limits.
2. Flow cytometry – CD-Chex plus BC is a sample of stabilised whole blood labelled with common leukocyte markers (CD11b, CD14 and CD16). This checks that flow cytometer results are within prescribed limits.
3. Sysmex (leukocyte count and differential subsets) – Eightcheck-3WP assay serves as a reliable control material for 3-part differential analysers monitoring up to 22 parameters and thus verifying analyser performance within prescribed limits.

Thus, sample analyses for all endpoint parameters will be fully validated and verifiable for accuracy.
Frozen plasma samples will be sent in batches to the pathology laboratory, Northern General Hospital, Sheffield Teaching Hospitals NHS Foundation Trust for analysis of plasma D-dimer levels by immunoturbidimetry [21].
Frozen plasma samples will be shipped to York Bioanalytical Solutions, York, for pharmacokinetic analyses:

1. Ticagrelor and ticagrelor active metabolite plasma concentrations using mass spectrometry [19]

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

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

- Ticagrelor 90 mg tablets
    - Brilique 90 mg tablets (AstraZeneca) will be obtained by STH NHS Foundation Trust pharmacy
    - Participants will be prescribed one 90-mg tablet each morning and one 90-mg tablet in the evening until discontinuation according to the randomised strategy
    - This medication may be prescribed by clinical staff outside the research team as part of usual care
- Aspirin 75 mg tablets (plain or dispersible)
    - Any available approved generic aspirin will be obtained by STH NHS Foundation Trust pharmacy

- Participants will be prescribed one 75-mg tablet each morning until discontinuation, if appropriate, according to the randomised strategy
- This medication may be prescribed by clinical staff outside the research team as part of usual care

8.2 Regulatory status of the drug

- Ticagrelor 90 mg twice daily is licensed in the UK, co-administered with aspirin, for the prevention of atherothrombotic events in adult patients with either acute coronary syndromes or a history of myocardial infarction and a high risk of developing an atherothrombotic event.
- Aspirin 75 mg OD (plain or dispersible tablets) is licensed in the UK for the following: secondary prevention of myocardial infarction; prevention of CV morbidity in patients suffering from stable angina pectoris; history of unstable angina pectoris, except during the acute phase; prevention of graft occlusion after Coronary Artery Bypass Grafting (CABG); coronary angioplasty, except during the acute phase; secondary prevention of TIA and ischaemic cerebrovascular accidents (CVA), provided intracerebral haemorrhages have been ruled out; acute myocardial infarction. Aspirin 75 mg OD is also licensed in the UK for the secondary prevention of thrombotic cerebrovascular or cardiovascular disease and following by-pass surgery.

8.3 Product Characteristics

Versions of the SmPCs approved by the MHRA for use in the trial will be used as reference safety information for ticagrelor 90 mg tablets and for aspirin 75 mg tablets. 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 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

Ticagrelor 90 mg tablets and aspirin 75 mg tablets will be sourced by NGH Pharmacy and supplied to the wards as per routine practice. Ticagrelor 90 mg tablets will be Brilique 90 mg (AstraZeneca). Aspirin 75 mg tablets will consist of any available approved generic preparation. Both ticagrelor and aspirin will be dispensed by ward staff from unsegregated clinical stock, as per standard practice. The investigators will ensure that instructions for when to discontinue ticagrelor and aspirin are recorded in the medical notes and relevant members of the clinical care team will be informed. The investigators will also ensure that planned discontinuation of ticagrelor and aspirin is recorded, as appropriate according to the randomised group, on the electronic prescribing system.

8.5 Preparation and labelling of Investigational Medicinal Product

Since the usual ward stock of ticagrelor and aspirin will be used, no study labelling will be used for the investigational medicinal product.

8.6 Dosage schedules

The dosage schedules are described in section 7.3, 7.7 and the trial flow chart within this protocol. Ticagrelor and aspirin will be administered, discontinued and/or modified as per standard of care other than as influenced by the randomisation strategy. If

discontinuation or dose modification is clinically indicated, other than as determined by the randomisation strategy, then this will go ahead and participants will be assessed against the withdrawal criteria described in section 7.10.
Ticagrelor will be taken in the morning and in the evening, approximately 12 hours apart, until discontinued according to the randomised treatment strategy. In the case of a missed dose of ticagrelor, the dose will be administered within 12 hours of the intended time if feasible, otherwise the participant will receive the next dose on time without taking a double dose. 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.
Aspirin will be taken in the morning either until the day before surgery (group A) or until the day of surgery (group B). In the case of a missed dose of aspirin, the dose will be administered within 24 hours of the intended time if feasible, otherwise the participant will receive the next dose on time without taking a double dose.

8.7 Dosage modifications

Discontinuation of ticagrelor or aspirin, other than as specified in the protocol, 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)

If dose modification is clinically indicated, then this will go ahead and participants will be assessed against the withdrawal criteria described in section 7.10.

8.8 Known drug reactions and interaction with other therapies

Details are to be found in section 8.9 below, and in the current SmPCs for ticagrelor and aspirin.

8.9 Concomitant medication

Recording of any prescribed or over-the-counter medication will be made at Visit 1, 2, 3, 4, 5 and 6. All individual medications, prescription and over-the-counter, will be recorded peri-event for any SAE or discontinuation due to AE. The following should be observed if any participant receives concomitant medication after enrolment:

Oral antiplatelet/anticoagulant therapies

Aspirin (acetylsalicylic acid): Aspirin use, with the exception of the study medication, is prohibited between Visit 1 and Visit 5. If no contraindication exists, paracetamol will be recommended if the need for analgesia or treatment of fever arises. If, between Visit 1 and Visit 5, a participant develops a clinical indication for and is administered a dose of aspirin other than the study regimen, they will be withdrawn from the study. Participants who receive at least one dose of IMP after randomisation will be followed up by telephone 30 ± 2 days after withdrawal.
Other oral thrombotic therapies: Treatment with any other oral antithrombotic therapy apart from the study medication (e.g., clopidogrel, prasugrel, dipyridamole, cilostazol, warfarin, dabigatran, rivaroxaban, edoxaban, apixaban) is prohibited between Visit 1 and Visit 5. However, if between Visit 1 and Visit 3, a participant develops a contraindication to aspirin or ticagrelor, these will be discontinued/withheld and they will be withdrawn from the study. Participants who receive at least one dose of IMP will be followed up by telephone 30 ± 2 days after withdrawal.

If regular treatment with such therapies becomes essential between Visit 1 and Visit 5 then participants will be withdrawn from the study. Participants who receive at least one dose of IMP will be followed up by telephone 30 ± 2 days after withdrawal.

Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs may affect the antiplatelet and immunomodulatory effects of aspirin whilst increasing the risk of gastric irritation/ulceration and renal impairment. NSAIDs may also increase the risk of bleeding in patients treated with ticagrelor. Levels of serum thromboxane B_2 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/COX2 inhibitor within 48 hours prior to visit 3, the participant will be withdrawn from the study unless CABG surgery is delayed and visit 3 is rescheduled with appropriate modification of the dosing strategy of the study medication (see section 7.10.1). Participants who receive at least one dose of IMP after randomisation will be followed up by telephone 30 ± 2 days after withdrawal.

Other anti-inflammatory/immunomodulatory drugs

Oral corticosteroids, disease-modifying anti-rheumatic drugs (including methotrexate at any dose), oral or subcutaneous immunosuppressants, and chemotherapy drugs: in the case of a participant requiring regular treatment with these agents between Visit 1 and Visit 5, they will be withdrawn unless CABG surgery is delayed until such time as the investigator believes the effects on inflammatory response will no longer be significant. Participants who receive at least one dose of IMP will be followed up by telephone 30 ± 2 days after withdrawal.
Treatment with topical or inhaled corticosteroids and oral or topical antihistamines is permitted.

Parenteral anticoagulants

Short-term treatment with parenteral anticoagulants (unfractionated heparin, low-molecular-weight heparin, bivalirudin, fondaparinux) is permitted unless the investigator deems this to be likely to have a substantial impact on the risk of perioperative bleeding, in which case the participant will be withdrawn from the study. Participants who receive at least one dose of IMP following randomisation will be followed up by telephone 30 ± 2 days after withdrawal.

Glycoprotein IIb/IIIa antagonists

Treatment with glycoprotein IIb/IIIa antagonists is permitted between Visit 1 and up to 4 hours before Visit 3. If a participant receives a glycoprotein IIb/IIIa antagonist less than 4 hours before CABG surgery, they will be withdrawn from the study. Participants who receive at least one dose of IMP following randomisation will be followed up by telephone 30 ± 2 days after withdrawal.

Fibrinolytic agents

Treatment with fibrinolytic agents should be avoided whenever possible during treatment with study medication. If a participant is to receive a fibrinolytic agent, then ticagrelor-treated patients should discontinue ticagrelor and subsequent treatment should be according to indication for fibrinolysis and standard local practice. Participants treated with a fibrinolytic agent will be withdrawn from the study. Participants who receive at

least one dose of IMP after randomisation will be followed up by telephone 30 ± 2 days after withdrawal.

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

P-glycoprotein interactions
Ticagrelor is a weak inhibitor of P-glycoprotein and consequently may increase concentrations of P-glycoprotein substrates including digoxin. The response to co-administration of ticagrelor and digoxin should therefore be observed carefully.

CYP3A4 interactions
Strong inhibitors of CYP3A4 substantially increase plasma ticagrelor levels whereas strong inducers of CYP3A4 substantially reduce plasma ticagrelor levels. 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 treatment with such therapies is essential in ticagrelor-treated patients, then ticagrelor should be discontinued and alternative treatment with either clopidogrel or prasugrel or no platelet $P2Y_{12}$ inhibitor should be considered according to standard local practice and the scheduled date of CABG surgery. If such therapy is prescribed following enrolment, the participant will be withdrawn from the study. Participants who receive at least one dose of IMP after randomisation will be followed up by telephone 30 ± 2 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 (i.e., doses of simvastatin or lovastatin ≤40 mg daily or any dose of any other statin is permitted). Standard monitoring of patients for possible statin-associated myopathy should be done.
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 also should be avoided (e.g., rifampin/rifampicin, rifabutin, phenytoin, carbamazepine, phenobarbital). If treatment with such therapies is essential in ticagrelor-treated patients then ticagrelor should be discontinued and alternative treatment with either clopidogrel or prasugrel or no platelet $P2Y_{12}$ inhibitor should be considered according to standard local practice and the scheduled date of CABG surgery. If such therapy is prescribed following enrolment, the participant will be withdrawn from the study. Participants who receive at least one dose of IMP after randomisation will be followed up by telephone 30 ± 2 days after withdrawal.

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

Other medications to be used with caution
In addition to those medications contraindicated or not recommended above, caution is advised in use of the following drugs in combination with aspirin, as detailed in the

current SmPC. Given participants will receive low-dose aspirin at 75 mg once-daily, there are no study-specific restrictions on prescription of these, but usual clinical considerations should be made.

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

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

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

- Ibuprofen: experimental data suggest that ibuprofen may inhibit the effect of low doses of aspirin on platelet aggregation in the case of concomitant administration. Nevertheless, the limitations of these data and uncertainties about the extrapolation of the data *ex vivo* to the clinical situation mean that it is impossible to draw definite conclusions about the regular use of ibuprofen. A clinically significant effect is considered unlikely where ibuprofen is used occasionally (for 48 hours or less).

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

- Phenytoin: the concomitant use of aspirin and phenytoin may lead to an increase in serum concentrations of phenytoin, but the unbound (therapeutic) concentration appears unaffected.

- Acetazolamide: caution is required when aspirin is administered concomitantly with acetazolamide owing to an increased risk of metabolic acidosis.

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

- Ciclosporin, tacrolimus: Concomitant use of NSAIDs and ciclosporin or tacrolimus may increase the nephrotoxic effect of ciclosporin and tacrolimus. The renal function should be monitored in case of concomitant use of these agents and aspirin.

8.10 Trial restrictions

It is intended that participants will remain in hospital from the time of enrolment until CABG surgery has been performed. Participants who are discharged before having CABG surgery will be withdrawn from the study. Participants who receive at least one dose of IMP after randomisation will be followed up by telephone 30 ± 2 days after withdrawal.

Surgery and other invasive procedures

If any surgery apart from planned CABG surgery or another invasive procedure becomes necessary during the study medication period, study medication will be handled according to routine clinical care.
The effects of aspirin 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 that leads to delay in planned CABG surgery, ticagrelor and aspirin will be continued at the discretion of the clinical care team as per routine clinical care.

8.11 Assessment of compliance with treatment

Compliance will be assessed through review of the hospital e-prescribing records and recording in the eCRF. Tablet counts will not be carried out since unsegregated ward stocks of ticagrelor and aspirin will be used.

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 ticagrelor • SmPC for aspirin

9.2 Operational definitions for (S)AEs

Adverse events and reactions, unless exempted, will be recorded and reported from the point of consent (visit 1) until visit 7, which will mark the end of the reporting period. The following (S)AEs are expected and will not be recorded or reported:

- Trivial bleeding that is not associated with clinically-relevant blood loss such as: spontaneous bruising or petechiae; prolonged bleeding from skin cuts/abrasions; needle puncture; or spontaneous mucocutaneous bleeding, such as gum bleeding.
- Post-operative bleeding that is considered usual by the clinical care team i.e. expected chest tube blood drainage
- Chest pain and/or shortness of breath consistent with cardiac ischaemia that resolves within 10 minutes of treatment with sublingual glyceryl trinitrate and is not associated with evidence of acute myocardial infarction
- Symptoms +/- signs of heart failure such as orthopnoea, paroxysmal nocturnal dyspnoea, shortness of breath on exertion, oedema, fatigue or malaise
- Tachycardia due to atrial fibrillation, non-sustained ventricular arrhythmia or sinus tachycardia unless due to acute blood loss pre-surgery
- Pyrexia not associated with evidence of allergic reaction
- Non-cardiac chest pain
- Other chest symptoms or reduction in lung capacity that are expected following CABG surgery
- Musculoskeletal pains
- Symptoms and/or clinical evidence of respiratory infection or other community-acquired or hospital-acquired infections, including sternal wound infection
- Headache
- Dizziness, faintness/presyncope or light-headedness not associated with acute blood loss
- Persistence or recurrence of symptoms that were present prior to the screening visit
- Deterioration in renal function attributable to X-ray contrast media or diuretic therapy
- Elevation in liver enzymes that was present prior to the screening visit or is <2x upper limit of reference range pre-surgery or any level following surgery
- <15% fall in any full blood count parameters pre-surgery or any fall in any full blood count parameters post surgery
- Any abnormality in coagulation parameters post surgery

The duration of follow-up period after last IMP administration is until 30 +/- 2 days following completion of surgery. Any carryover effects from IMPs will be detectable within this window given that the effects of ticagrelor last approximately 5 days[24] and the effects of aspirin last for the duration of the life of platelets (approximately 10 days)[25]. All SAEs will be reported to the Sponsor within 24 hours of the research team becoming aware of them. This will be using the Sponsor's proforma which will be sent by email to a

dedicated address provided specifically for this purpose. A copy will be kept in the trial master file and the AE log will be completed. All SAEs will be reviewed at the regular trial management group meetings.
All other AEs not meeting the criteria for reporting as serious will be recorded in the CRF. The investigators will assess the relatedness of adverse events to the IMPs (ticagrelor, aspirin).

9.3 Recording and reporting of SAEs, SARs AND SUSARs

SAEs, unless exempted as above, and SUSARs will be recorded and reported from the point of consent (Visit 1) until Visit 7, which will mark the end of the reporting period.

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, ongoing status, 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 expected.

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 causality (and expectedness if causal) has been assessed and the event has resolved or a final outcome has been reached.
All SAEs assigned by the PI or delegate as both suspected to be related to IMP-treatment and unexpected will be classified as SUSARs and will be subject to expedited reporting to the Medicines and Healthcare Products Regulatory Agency (MHRA). The Sponsor will inform the MHRA, the REC and the relevant Marketing Authorisation Holder(s) of SUSARs within the required expedited reporting timescales.

9.4 Responsibilities

Principal Investigator (PI) or delegate:

1. Collection and verification of AEs and ARs.
2. Using medical judgement in assigning seriousness, causality and whether the event/reaction was expected using the Reference Safety Information approved for the trial.
3. Ensuring that all SAEs are recorded and reported to the Sponsor within 24 hours of becoming aware of the event and provide further follow-up information as soon as available. Ensuring that SAEs are chased with Sponsor if a record of receipt is not received within 2 working days of initial reporting.
4. Ensuring that AEs and ARs are recorded and reported to the Sponsor in line with the requirements of the protocol.
5. Preparing standard tables and other relevant information for the DSUR in collaboration with the CI.

Chief Investigator:

1. Clinical oversight of the safety of patients participating in the trial, including an ongoing review of the risk / benefit.
2. Immediate review of all SUSARs.

3. Assigning Medical Dictionary for Regulatory Activities (MedDRA) or Body System coding to all SAEs and SARs.
4. 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 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. Ensuring timely review of the drafted DSUR and submission to the MHRA and REC.

9.5 Notification of deaths

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

9.6 Pregnancy reporting

- Pregnancy is not expected to occur as women of childbearing potential who are pregnant or unwilling to use reliable contraception will be excluded from study involvement. Nevertheless, any pregnancy occurring in a study participant should be reported to the CI 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 ward staff comment.
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 events 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 REC 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.

9.11 Notification of device-related adverse events

Any AEs related to the use of the CytoSorb device within its CE-marked indication within the protocol will be notified to CytoSorbents and, if appropriate, to the MHRA via the Yellow Card scheme. The research team will provide any necessary support and anonymous information to CytoSorbents in order to appropriately classify the AE in terms of severity, expectedness and relatedness to the CytoSorb device.

10 STATISTICS AND DATA ANALYSIS

10.1 Sample size calculation

Standard-of-care management of ticagrelor in our institution involves stopping ticagrelor 5 or more days prior to CABG surgery (unless platelet aggregation studies indicate it is safe to proceed at 4 days after stopping ticagrelor). Aspirin is continued without interruption, including on the morning of surgery. Our recent unpublished platelet aggregation studies of patients receiving this standard-of-care management indicate that platelet aggregation responses to collagen 4 ug/mL, assessed using LTA, recover following cessation of ticagrelor to mean 70.6% (SD 11.1) but then, as a consequence of platelet activation during CPB leading to platelet desensitization, decline to mean 44.3% (SD 19.3) immediately post-CPB (Figure 4). Testing for normal distribution indicates that the data at V3 pass various normality tests (e.g., Shapiro-Wilk test P value = 0.9142).

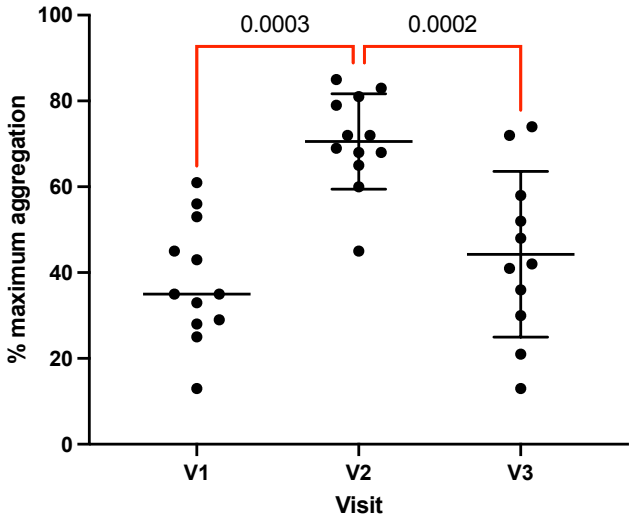

Figure 4. Platelet aggregation induced by collagen 4 ug/mL during treatment with aspirin and ticagrelor ('V1'), immediately before CABG surgery ('V2') and immediately after cardiopulmonary bypass ('V3').

During treatment with both ticagrelor and aspirin, the platelet aggregation responses to collagen 4 ug/mL showed a mean of 38.0% (SD 14.1). According to the experimental arm of the proposed trial prior to 12 May 2023, patients will receive last doses of ticagrelor and aspirin approximately 24 hours before undergoing CABG surgery. Our previous studies indicated that there will be a small increase in collagen-induced platelet aggregation related to reduction in plasma ticagrelor level at this time point (unpublished data) and also a significant increase of approximately 20% due to offset of the effect of aspirin (Parker WA et al. Platelets 2019), since collagen-induced platelet aggregation is sensitive to aspirin dose effect (Figure 5). Review of the data from the first 10 randomised patients undergoing CABG surgery indicated less increase in collagen-induced platelet aggregation than that predicted associated with increased bleeding and so the trial was temporarily halted on 12 May 2023 and the protocol revised. For the experimental arm according to the revised protocol dated 19 May 2023, patients will receive last doses of ticagrelor and aspirin approximately 48 hours before undergoing CABG surgery (unless surgery is postponed by one day in which case they will receive a loading dose of aspirin 300mg approximately 24 hours before undergoing CABG surgery having discontinued ticagrelor and aspirin for the previous 48 hours).

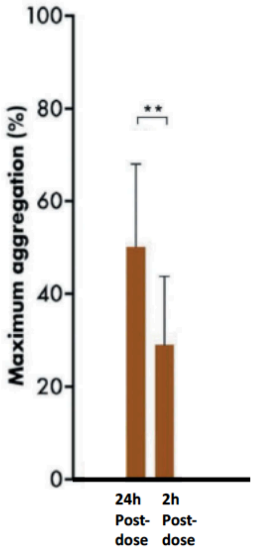

Figure 5. Platelet aggregation induced by collagen 4 ug/mL in ACS patients treated with ticagrelor and aspirin 75mg once daily at 24 hours post-aspirin (trough) and 2 hours post-aspirin (peak).
Reproduced from Parker WA et al, Platelets 2019 (https://doi.org/10.1080/09537104.2019.1572880)

Consequently, it is expected that pre-surgery levels of collagen-induced platelet aggregation in the experimental arm will be similar to those seen post-CPB in the standard-of-care arm. The experimental arm will then benefit from two mechanisms for preserving platelet reactivity during CPB: firstly, CytoSorb will progressively remove the ticagrelor during CPB in order to reverse its effects (although, due to its large volume of distribution, some ticagrelor is expected to remain leading to residual platelet inhibition); secondly, based on numerous previous studies, the presence of ticagrelor at therapeutic concentrations for at least some of the CPB will inhibit many of the processes that lead to the platelet activation and subsequent desensitization seen in the standard-of-care arm. This provides a rationale for non-inferiority of the experimental approach.
The initial power calculation was performed as follows: Based on the previously-observed SD (19.3%) of collagen-induced platelet aggregation in the standard-of-care patients, then, if the experimental strategy is truly as effective as the standard strategy in preserving post-operative platelet reactivity, 36 patients (18 patients per group) are required to be 90% sure that the lower limit of a one-sided 97.5% confidence interval (alpha of 0.025) will be above a non-inferiority margin of -21.5% (determined using PASS 2021, v21.0.1; see Appendix B). 40 patients will be required to allow for 10% drop-out/missing data in each arm. Patients enrolled but not proceeding to CABG surgery will be replaced. Following review of the data on the first 10 randomised patients undergoing CABG surgery, the study was temporarily halted due to less than expected recovery in platelet inhibition in the experimental arm following surgery and associated bleeding events. A revised power calculation was then performed as follows for patients enrolled under the revised protocol dated 29 May 2023 to be assessed as a separate cohort to the patients enrolled prior to 12 May 2023: Based on the previously-observed SD (19.3%) of collagen-induced platelet aggregation in the standard-of-care patients, then, if the experimental strategy is truly as effective as the standard strategy in preserving post-operative platelet reactivity, 28 patients (14 patients per group) are required to be 80% sure that the lower limit of a one-sided 97.5% confidence interval (alpha of 0.025) will be above a non-inferiority margin of -21.5% (determined using SAS software version 9.4; see Appendix C). 30 patients will be required to allow for drop-out/missing data for 1

patient in each arm. Patients enrolled but not proceeding to CABG surgery and undergoing at least either Visit 4 or Visit 5 will be replaced. Based on similar values for collagen-induced platelet aggregation between Visit 4 and Visit 5, as well as failure of LTA in some cases due to haemolysis induced by cardiopulmonary bypass, if there is failure to obtain LTA data at Visit 4 then the value for collagen-induced platelet aggregation at Visit 5 may be used as an estimation of the Visit 4 value.

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:

At visit 1

- Demographic data (age, sex, race/ethnicity)
- Vital signs (pulse, blood pressure and temperature)
- Weight and BMI
- Results of routine laboratory tests (full blood count, urea & electrolytes, liver function tests, clotting screen
- Results of routine coronary angiogram (number and location of major coronary arteries with stenosis >50%)
- Results of routine ECGs (presence of pathological T wave inversion, presence of ischaemic ST changes, presence of pathological Q waves indicating Q-wave myocardial infarction)

At visit 2

- Vital signs: pulse, blood pressure and temperature
- Concomitant medication
- Platelet function test results (LTA responses to collagen 4 and 16 ug/mL, AA 1 mmol/L, ADP 5 and 20 umol/L, 5-HT 1 umol/L + epinephrine 10 umol/L, TRAP 8 and 32 umol/L; ADP-induced P-selectin expression; Multiplate ADPtest and TRAP test AUC; VerifyNow $P2Y_{12}$ PRU; VASP PRI; serum thromboxane B_2)
- Inflammatory parameters (plasma IL-6; plasma TNF-α; circulating leukocyte count plus subsets; platelet-leukocyte aggregates; monocyte and neutrophil CD11b expression)
- Fibrin clot dynamics (lag time, lysis time, final clot turbidity)
- Plasma D-dimer level
- Haemostasis (bleeding time)
- Pharmacokinetics (plasma levels of ticagrelor and its active metabolite)

- Haematocrit
- Platelet count

Categorical data will be reported as frequency and percentages. Continuous data will be reported as number, mean, standard deviation, minimum and maximum, with 95% confidence interval as specified, if normally distributed, otherwise using median and interquartile range. Logarithmic transformation will be considered for analysis purposes if data are skewed. Differences between the two groups will be assessed using independent samples T-tests and analysis of variance (ANOVA) for continuous variables meeting normality assumptions, and using Wilcoxon rank-sum tests and Kruskal-Wallis one-way analysis of variance when normality assumptions are not met. Inferential statistical tests will be two-sided, using alpha (α) of 0.05 as the critical value, unless specified otherwise.
A CONSORT flow diagram will be prepared for inclusion in the report of study findings.

10.3.2Primary outcome analysis

The primary endpoint will be % platelet aggregation induced by collagen 4 ug/mL immediately post-CPB (or at 2 hours post-surgery if the value post-CPB is unavailable), assessed using LTA. Data will be tested for normality using the Shapiro-Wilk test. Confidence intervals of the mean platelet aggregation response will be calculated. Non-inferiority will be assumed if the lower confidence limit for the experimental group (group A) is less than 21.5% below the mean platelet aggregation response in the standard-of-care group (group B), with significance assessed using a non-inferiority t-test. Between group differences will also be analysed by an independent samples T-test or, for non-parametric data, using a Wilcoxon rank-sum test.

10.3.3Secondary outcome analysis

When comparing the experimental group (group A) to the standard-of-care group (group B), the following will be assessed:

1. Absolute LTA response to AA 1 mmol/L immediately post-CPB using an independent samples T-test.
2. VerifyNow $P2Y_{12}$ assay PRU immediately post-CPB using an independent samples T-test.
3. Increase in plasma IL-6 concentration from pre-surgery to immediately post-CPB using an independent samples T-test.
4. Increase in plasma D-dimer concentration from pre-surgery to immediately post-CPB using an independent samples T-test.
5. Absolute skin bleeding time immediately post-surgery using an independent samples T-test.
6. Total chest tube drainage from immediately post-surgery to 24 hours post-surgery using an independent samples T-test.

When assessing the specific effect of the CytoSorb device on ticagrelor and ticagrelor active metabolite plasma levels, the following will be assessed independently in each group:

1. Change in plasma ticagrelor concentration from pre-surgery to immediately post-CPB using a paired samples T-test.
2. Change in plasma ticagrelor active metabolite concentration from pre-surgery to immediately post-CPB using a paired samples T-test.

10.3.4Tertiary outcome analysis

When comparing the experimental group (group A) to the standard-of-care group (group B), the following will be assessed:

1. Absolute LTA response to collagen 4 ug/mL pre-surgery using an independent samples T-test.
2. Change in LTA response to collagen 4 ug/mL from pre-surgery to immediately post-CPB using an independent samples T-test.
3. Absolute LTA response to AA 1 mmol/L pre-surgery using an independent samples T-test.
4. Change in LTA response to AA 1 mmol/L from pre-surgery to immediately post-CPB using an independent samples T-test.
5. Absolute LTA responses to collagen 16 ug/mL, ADP 5 and 20 umol/L, 5-HT 1 umol/L + epinephrine 10 umol/L combination, and thrombin-receptor-activating peptide (TRAP) 8 and 32 umol/L pre-surgery using an independent samples T-test.
6. Absolute LTA responses to collagen 16 ug/mL, ADP 5 and 20 umol/L, 5-HT 1 umol/L + epinephrine 10 umol/L combination, and thrombin-receptor-activating peptide (TRAP) 8 and 32 umol/L immediately post-CPB using an independent samples T-test.
7. Change in LTA responses to collagen 16 ug/mL, ADP 5 and 20 umol/L, 5-HT 1 umol/L + epinephrine 10 umol/L combination, and thrombin-receptor-activating peptide (TRAP) 8 and 32 umol/L from pre-surgery to immediately post-CPB using an independent samples T-test.
8. Absolute VerifyNow PRU values pre-surgery using an independent samples T-test.
9. Change in VerifyNow PRU values from pre-surgery to immediately post-CPB using an independent samples T-test.
10. Absolute VerifyNow % inhibition values pre-surgery using an independent samples T-test.
11. Absolute VerifyNow % inhibition values immediately post-CPB using an independent samples T-test.
12. Change in VerifyNow % inhibition values from pre-surgery to immediately post-CPB using an independent samples T-test.
13. Absolute Multiplate ADPtest AUC values pre-surgery using an independent samples T-test.
14. Absolute Multiplate ADPtest AUC values immediately post-CPB using an independent samples T-test.
15. Change in Multiplate ADPtest AUC values from pre-surgery to immediately post-CPB using an independent samples T-test.
16. Absolute Multiplate TRAPtest AUC values pre-surgery using an independent samples T-test.
17. Absolute Multiplate TRAPtest AUC values immediately post-CPB using an independent samples T-test.
18. Change in Multiplate TRAPtest AUC values from pre-surgery to immediately post-CPB using an independent samples T-test.
19. Absolute VASP PRI values pre-surgery using an independent samples T-test.
20. Absolute VASP PRI values immediately post-CPB using an independent samples T-test.
21. Change in VASP PRI values from pre-surgery to immediately post-CPB using an independent samples T-test.
22. Absolute serum thromboxane B_2 levels, assessed using ELISA, pre-surgery using an independent samples T-test.
23. Absolute serum thromboxane B_2 levels, assessed using ELISA, immediately post-CPB using an independent samples T-test.
24. Change in serum thromboxane B_2 levels, assessed using ELISA, from pre-surgery to immediately post-CPB using an independent samples T-test.
25. Absolute unstimulated platelet P-selectin expression immediately post-CPB using an independent samples T-test.
26. Change in unstimulated platelet P-selectin expression from pre-surgery to immediately post-CPB using an independent samples T-test.
27. Absolute platelet P-selectin expression induced by ADP 30 umol/L pre-surgery using an independent samples T-test.
28. Absolute platelet P-selectin expression induced by ADP 30 umol/L immediately post-CPB using an independent samples T-test.

29. Change in platelet P-selectin expression induced by ADP 30 umol/L from pre-surgery to immediately post-CPB using an independent samples T-test.
30. Increase in plasma TNF-α level, assessed using ELISA, from pre-surgery to immediately post-CPB using an independent samples T-test.
31. Increase in unstimulated neutrophil CD11b expression, assessed using flow cytometry, from pre-surgery to immediately post-CPB using an independent samples T-test.
32. Increase in unstimulated monocyte CD11b expression from pre-surgery to immediately post-CPB using an independent samples T-test.
33. Increase in unstimulated platelet-monocyte aggregates from pre-surgery to immediately post-CPB using an independent samples T-test.
34. Increase in unstimulated platelet-neutrophil aggregates from pre-surgery to immediately post-CPB using an independent samples T-test.
35. Absolute platelet-monocyte aggregates stimulated by ADP 30 umol/L pre-surgery using an independent samples T-test.
36. Absolute platelet-monocyte aggregates stimulated by ADP 30 umol/L immediately post-CPB using an independent samples T-test.
37. Absolute platelet-neutrophil aggregates stimulated by ADP 30 umol/L pre-surgery using an independent samples T-test.
38. Absolute platelet-neutrophil aggregates stimulated by ADP 30 umol/L immediately post-CPB using an independent samples T-test.
39. Change in fibrin lag time from pre-surgery to immediately post-CPB using an independent samples T-test.
40. Change in fibrin clot lysis time from pre-surgery to immediately post-CPB using an independent samples T-test.
41. Change in fibrin clot maximum turbidity from pre-surgery to immediately post-CPB using an independent samples T-test.
42. Absolute skin bleeding time pre-surgery using an independent samples T-test.
43. Change in skin bleeding time from pre-surgery to immediately post-surgery using an independent samples T-test.
44. Absolute plasma ticagrelor concentration pre-surgery using an independent samples T-test.
45. Absolute plasma ticagrelor concentration immediately post-CPB using an independent samples T-test.
46. Absolute plasma ticagrelor active metabolite concentration pre-surgery using an independent samples T-test.
47. Absolute plasma ticagrelor active metabolite concentration immediately post-CPB using an independent samples T-test.
48. Change in whole blood platelet count from pre-surgery to immediately post-CPB using an independent samples T-test.
49. Change in haematocrit from pre-surgery to immediately post-CPB using an independent samples T-test.
50. Change in whole blood total leukocyte and differential counts from pre-surgery to immediately post-CPB using an independent samples T-test.
51. Total number of units of blood products and type transfused from pre-surgery to 24 hours post-surgery using an independent samples T-test.
52. Total length in days of hospital stay, time in days from coronary angiography to CABG surgery, time in days from randomisation to CABG surgery, length of stay in days from randomisation to hospital discharge, and length of stay in days on intensive care unit using an independent samples T-test.

In addition, the following will be presented descriptively:

1. Measurements taken at randomisation and the changes from randomisation to immediately pre-surgery.
2. Measurements taken at 2 hours post-surgery and the changes from pre-surgery or immediately post-CPB/post-surgery to 2 hours post-surgery.

3. The frequency and percentage of patients with thrombotic and/or bleeding events occurring between randomisation and CABG surgery
4. The frequency and percentage of patients requiring reoperation for bleeding after CABG surgery.

Safety endpoints
Incidence (number and percent of subjects) will be summarized for AEs, ARs, SAEs, SARs, and SUSARs. Only treatment-emergent events occurring on or after first dose of study drug will be summarized, using the safety analysis set (see Section 10.7).

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 may be performed.

10.6 Interim analysis and criteria for the premature termination of the trial
No interim analyses are planned.

10.7 Participant analysis populations
- The pharmacodynamic and pharmacokinetic analysis set will include all participants who are randomised and proceed to CABG surgery during the study.
- 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 study medication following randomisation.

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 an independent samples T-test or ANOVA, missing data will be estimated by multiple imputation using the IBM SPSS software package. Sensitivity analyses (without imputation) will be carried out to report the robustness of this approach.

10.9 Other statistical considerations.
Not applicable to this study

11 DATA MANAGEMENT

11.1 Data collection tools and source document identification

Source data will be recorded in the following locations depending on the nature of the data: in the paper medical records; on the hospital electronic records systems; on machine print-outs for some of the laboratory assays; and on the study-specific paper case report form (CRF). Specific arrangements for recording source data will be described in the management arrangements form. Source data will feed from paper medical records, hospital electronic records systems and machine print-outs for some of

the laboratory assays to the study-specific paper CRF. Data from the study-specific CRF will then be entered into the trial master database via data entry personnel on a regular basis during conduct of the trial.
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 sequential numeric participant identification code (e.g., “001”, “002”, etc.) that allows identification of all the data reported for each participant. All electronic data will be kept securely stored on University of Sheffield systems, or external handlers contracted by the University of Sheffield for this purpose. The Sponsor will be responsible for ensuring compliance with the requirements outlined above.

11.3 Access to Data

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

11.4 Archiving

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

12 MONITORING, AUDIT & INSPECTION

- A Trial Monitoring Plan will be put in place by the Sponsor based on the trial risk assessment, which may include on-site monitoring. Sponsor monitoring will focus on compliance with GCP and will be complemented by internal monitoring by the research team headed by the CI, who will be responsible for verification of the accuracy of study data.
- The study team will confirm the accuracy of CRF entries against source data and will keep records of this internal monitoring.
- It is anticipated that Sponsor monitoring 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 Sponsor monitoring personnel will be determined by the Sponsor. The processes reviewed will include participant enrolment, consent, eligibility, and allocation to trial groups; adherence to trial interventions and policies to protect participants, including reporting of harm and completeness and timeliness of data collection.
- The research team will keep records of internal monitoring which will verify accuracy of study data entered into the study database.

- Monitoring may be performed remotely or may be through site visit to review original documentation.

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 will be reviewed and approved by scientific, medical and statistics personnel of Cytosorbents Inc. In addition, an independent peer review of the statistical aspects of the study will be conducted.

13.3 Public and Patient Involvement

Members of the Sheffield Cardiovascular Patient Panel will be involved in reviewing the design and content of the patient documentation.

13.4 Regulatory Compliance

- The trial will not commence until a Clinical Trial Authorisation (CTA) is obtained from the MHRA and a Favourable REC opinion is provided.
- 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 or may apply 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 within the Trust to avoid any data breaches. Transmission of information relating to safety e.g., 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/principal investigator and co-investigators

Robert F. Storey: institutional research grants/support from AstraZeneca, Cytosorbents, GlyCardial Diagnostics and Thromboserin; consultancy fees from Alnylam, AstraZeneca, Bayer, Bristol Myers Squibb/Pfizer alliance, CSL Behring, Cytosorbents, GlyCardial Diagnostics, Hengrui,

Idorsia, Novartis, PhaseBio, Portola, Sanofi Aventis and Thromboserin; speaker fees from AstraZeneca, Bayer, Bristol Myers Squibb/Pfizer alliance and Intas Pharmaceuticals.

Steven Hunter, Nadir Elamin, William A. E. Parker: no conflicts of interest.

13.9 Indemnity

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

13.10 Amendments

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

13.11 Post-trial care

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

13.12 Access to the final trial dataset

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

14 DISSEMINATION POLICY

14.1 Dissemination policy

- The data arising from the trial will be owned by University of Sheffield.
- On completion of the trial, the data will be analysed and tabulated and a Final Trial Report prepared.
- The trial report will be accessible via online registries in which the trial will be listed, in a medical journal and on request from the CI.
- The CI will retain the sole right to publish any of the trial data.
- Funding by Cytosorbents will be acknowledged within any publications. Cytosorbents will review and comment on any manuscript prior to submission for publication but will not have publication rights of the data from the trial.
- Participants will be able to access a summary of the results via the University of Sheffield website.
- It will not be possible for the participant to specifically request results from the investigators.
- The disclosure of the trial protocol, full trial report, anonymised participant level dataset, and statistical code for generating the results may be made available to interested parties at the discretion of the CI, not before 1 year after study completion.

14.2 Authorship eligibility guidelines and any intended use of professional writers

Those individuals who contribute significantly to the development, conduct and writing up of the study will be offered authorship of the final trial report. Individually-named authors will meet the

authorship criteria of The International Committee of Medical Journal Editors. No professional medical writers will be involved in the preparation of reports or publications.

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APPENDIX A: Sheffield Teaching Hospitals NHS Foundation Trust protocol for platelet function testing prior to cardiac surgery

Pre-operative platelet function testing in patients who have taken a $P2Y_{12}$ inhibitor within 5-7 days prior to cardiac surgery

The European Society of Cardiology (ESC) recommends delaying CABG surgery, when feasible, until 5 days after the last dose of ticagrelor or clopidogrel and 7 days after the last dose of prasugrel.[26] However, the offset of effect of these drugs is variable[27] and ESC now endorses shorter delays to surgery if platelet function testing using a validated method, such as the Multiplate system, indicates this is appropriate.[28] The following protocol is based on best available evidence for the use of Multiplate for determining the level of $P2Y_{12}$ inhibition:

Only perform Multiplate ADPtest in hirudin sample tubes according to manufacturer's instructions

ADPtest result (AUC)	*Interpretation*	*Suggested action*
> 50	**No evidence of $P2Y_{12}$ inhibition**	**Proceed to surgery**
30-50	**Possible $P2Y_{12}$ inhibition**	**Consider retesting after 24 h**
<30	**Significant $P2Y_{12}$ inhibition**	**Consider retesting after 48 h or use standard time delay**

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APPENDIX B: Sample size calculation method

PASS 2021, v21.0.1 (courtesy of Hans Kroger, CytoSorbents Inc)

Two-Sample T-Tests for Non-Inferiority Assuming Equal Variance
Numeric Results for an Equal-Variance T-Test

δ = μ1 - μ2 = μT - μR
Hypotheses: H0: δ ≤ -NIM vs. H1: δ > -NIM

Power	N1	N2	N	-NIM	δ	σ	Alpha
0.89545	18	18	36	-21.3	0	19.3	0.025
0.89816	18	18	36	-21.4	0	19.3	0.025
0.90082	18	18	36	-21.5	0	19.3	0.025
0.90342	18	18	36	-21.6	0	19.3	0.025

Report Definitions
Power is the probability of rejecting a false null hypothesis. N1 and N2 are the number of items sampled from each population.
N = N1 + N2 is the total sample size. -NIM is the magnitude and direction of the margin of non-inferiority. Since higher means are better, this value is negative and is the distance below the reference mean that is still considered non-inferior.
δ = μ1 - μ2 = μT - μR is the difference between the treatment and reference means at which power and sample size calculations are made. σ is the assumed population standard deviation for each of the two groups. Alpha is the probability of rejecting a true null hypothesis.

Summary Statements
Group sample sizes of 18 and 18 achieve 90% power to detect non-inferiority using a one-sided, two-sample equal-variance t-test. The margin of non-inferiority is -21.3. The actual difference between the means is assumed to be 0. The significance level (alpha) of the test is 0.025. The data are drawn from populations with a standard deviation of 19.3 in both groups.

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APPENDIX C: Sample size calculation for protocol v3.0, 19 May 2023

Software package SAS (v9.4) was used to perform the sample size calculation, as follows:

The POWER Procedure
Two-Sample t Test for Mean Difference

Fixed Scenario Elements	
Distribution	Normal
Method	Exact
Number of Sides	U
Null Difference	-21.5
Alpha	0.025
Mean Difference	0
Standard Deviation	19.3
Group 1 Weight	1
Group 2 Weight	1

Computed N Total			
Index	Nominal Power	Actual Power	N Total
1	0.75	0.778	26
2	0.80	0.810	28
3	0.85	0.862	32

Consequently 14 patients per group are required to provide at least 80% power with alpha of 0.025.