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Ticagrelor in Remote Ischemic Preconditioning (TRIP) Study

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Sponsor: ***Hellenic Society of Interventional Cardiology***

CONFIDENTIAL

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this study Clinical Study Protocol.

Abbreviation or special term	**Explanation**
ACS	Acute Coronary Syndrome
CAD	Coronary Artery Disease
CNT2/3	Concentrative Nucleoside Transporters
CRUSADE	Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the ACC/AHA guidelines
EMA	European Medicines Agency
ENT 1/2	Equilibrative Nucleoside Transporters
ESC	European Society of Cardiology
GCP	Good Clinical Practice
IPC	Ischemic Preconditioning
I/R	Ischemia/ Reperfusion
MI	Myocardial Infarction
NSTE-ACS	non ST-segment elevation Acute Coronary Syndrome
PCI	Percutaneous Coronary Intervention
PMI	Periprocedural Myocardial Injury
RIPC	Remote Ischemic Preconditioning
STEMI	ST-segment elevation MI

1. INTRODUCTION

1.1 Background

Remote Ischemic Preconditioning in PCI

Even though technical advances in percutaneous coronary intervention (PCI), over the past two decades, have resulted in a safe procedure with minimal complications, in several patients the procedure is complicated by peri-procedural injury, detected by elevated values of biomarkers of myocardial necrosis. According to the biomarker used, the incidence of peri-procedural myocardial injury in elective procedures ranges between 20% and 45%, with widespread adoption of troponin tests allowing detection of smaller amounts of necrosis.[1, 2] Peri-procedural cardiac troponin elevation has been associated with new irreversible myocardial injury, detected by delayed-enhancement magnetic resonance imaging,[3] and even though the prognostic significance of peri-procedural CK-MB and cardiac troponin elevation has been highly debated, several studies have reported that peri-procedural injury is associated with worst prognosis.[4, 5]

Peri-procedural myocardial injury may be categorized according to its pathogenesis into two types.[2] Type 1 or proximal type of peri-procedural myocardial injury is mainly attributed to side branch occlusion during balloon inflation or stent deployment. Side branch occlusion, occurring in 12.5-19% of cases, is mainly caused by plaque shift, thrombus formation, or dissection at the takeoff of the side branch. Type 2 or distal type of peri-procedural myocardial injury, which accounts for 50-75% of all cases, results from distal embolism of atheromatous material, platelet activation and thrombosis precipitating microvascular plugging, neurohormonal activation and modulation of vascular and myocardial functions, and oxidative stress with inflammation.

If peri-procedural injury incidence could be attenuated, then clinical outcomes of stable CAD following PCI may be expected to improve. One approach to reducing myocardial injury is through cardioprotective intervention.[2] Conditioning the myocardium to protect against procedural ischemia/ reperfusion (I/R) injury is such an intervention used in both the experimental and clinical setting.[6] Conditioning refers to an intrinsic myocardial mechanism of cardioprotection triggered by inducing brief, sub-lethal episodes of ischemia and reperfusion.[6] Conditioning can be defined according to the temporal relationship between the conditioning intervention and onset of the ischemic insult - conditioning before the sustained insult is preconditioning, conditioning during the sustained insult is perconditioning, whilst conditioning after the sustained insult is postconditioning.[7] Furthermore, preconditioning of the myocardium can be achieved from a remote organ, via a non-invasive approach.[7] Here, focus is on remote ischemic preconditioning (RIPC) and its potential effect to reduce myocardial I/R injury induced during PCI, thus improving clinical outcomes of elective PCI.

Mechanism of preconditioning and the role of adenosine

The mechanism behind the protective effects of RIPC in the setting of PCI has been recently reviewed by our group.[8] Mechanistically, preconditioning is best considered in terms of triggers, mediators, and effectors.[6, 9] The activators of the preconditioning cascade are termed “triggers” and are broadly extracellular receptor/ligand interactions with autocoid, endocrine or paracrine signaling molecules. The ischemic conditioning signal is a summation of signals

derived from multiple disparate receptor/ligand interactions, which reaches a threshold once sufficient combined signals are generated.[6, 9]

Among the most well-established preconditioning triggers, are adenosine, bradykinin and opioids. Adenosine is thought to be crucial in initiating the preconditioning cascade.[10] Activation of the G-coupled adenosine A1 receptor triggers IPC protection, whereas adenosine receptor antagonists can block IPC protection.[11] In a similar manner, bradykinin infusion induces cardioprotection which can be abolished by bradykinin receptor blockers.[12]

Adenosine is a purine nucleoside produced primarily through the metabolism of ADP, and its plasma levels increase after cellular stresses such as injury, ischemia/reperfusion, or inflammation.[13] Adenosine is rapidly taken up by cells through sodium-independent equilibrative nucleoside transporters (ENT 1/2) and sodium-dependent concentrative nucleoside transporters (CNT 2/3).[14]

Intracellular adenosine is metabolized to inosine by adenosine deaminase or transformed into adenine nucleotides by adenosine kinase.[15] Because of its rapid cellular uptake and metabolism, extracellular adenosine has a half-life of a few seconds, which can be prolonged by inhibition of its transport into cells.[16]

Several studies provide evidence that ticagrelor inhibits cellular uptake of adenosine.[14, 17, 18] Ticagrelor inhibited adenosine uptake by washed human erythrocytes and by human, dog, and rat cell lines. Considering that the experiments were performed under sodium-free conditions and with cell lines that express ENT1 but not ENT2, it was assumed that ticagrelor inhibits sodium-independent ENT1.[17] The identity of the target transporter was recently confirmed with cells transfected with human transporters (ENT1, ENT2,CNT2, and CNT3). In these experiments, ticagrelor significantly inhibited adenosine uptake only in cells that expressed ENT1 .[14]

1.2 Research hypothesis

We will study the effect of ticagrelor on the reduction of PMI and the incidence of post-PCI MI with the use of RIPC. Our primary research hypothesis is that treatment with ticagrelor will potentiate the effects of RIPC and, therefore, reduce PMI, as assessed by post-procedural troponin release.

1.3 Rationale for conducting this study

Our research group has significant experience in utilizing RIPC for the prevention of periprocedural myocardial injury (PMI) in the setting of PCI.[8] We have recently demonstrated in a randomized clinical trial that RIPC can effectively reduce PMI and post-PCI myocardial infarction in the setting of ad hoc PCI for stable coronary artery disease.[19] Furthermore, we have recently published a meta-analysis, providing compelling evidence for the beneficial effects of RIPC in the setting of elective PCI.[20].

In this setting we have designed a randomized clinical trial to assess the effect of ticagrelor on the reduction of PMI and the incidence of post-PCI MI with the use of RIPC.

1.4 Benefit/risk and ethical assessment

Peri-procedural cardiac troponin elevation has been associated with new irreversible myocardial injury and several studies have reported that peri-procedural injury is associated with worst prognosis. According to the available evidence, the prognostic significance of PMI is more pronounced when pre-procedural troponin is increased, i.e. in the setting of acute coronary syndromes. Data from the Cardiac Remote Ischemic Preconditioning in Coronary Stenting (CRISP Stent) study support that implementation of a RIPC protocol during PCI may reduce major adverse cardiovascular events during long-term follow-up and that post-procedural troponin I concentration associated with the development of major adverse cardiovascular events.[21] If this protective effect is potentiated with treatment with ticagrelor, patients are expected to further benefit.

Ticagrelor has been shown to reduce significantly the rate of death from vascular causes, myocardial infarction, or stroke in patients with an acute coronary syndrome (ACS) with or without ST-segment elevation, compared to clopidogrel. These benefits were obtained without an increase in the rate of overall major bleeding but with an increase in the rate of non-procedure-related bleeding.[22] Accordingly, treatment with ticagrelor in patients undergoing PCI for non ST-segment elevation ACS (NSTE-ACS) or ST-segment elevation MI (STEMI) has been approved by the European Medicines Agency (EMA) and carries a Class I level of evidence B recommendation from the European Society of Cardiology (ESC)[23]

Even though ticagrelor has not received approval in patients with stable CAD, several clinical trials have assessed its pharmacodynamic and pharmacokinetic effects in comparison with clopidogrel in this patient population, proving superior efficacy in platelet inhibition with a safety profile consistent with previous studies.[24,25,26,27]

In treating patients with stable CAD with a more potent antiplatelet drug, lies a risk of increased bleeding, even though according to previous studies this is expected to be minor and non-life threatening (PLATO).[22] To minimize patient risk, patients with a CRUSADE [Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the ACC/AHA guidelines] bleeding risk score >50 will be excluded from the study. Furthermore, duration of ticagrelor treatment will be kept minimal (1 month). Furthermore, since use of ticagrelor is not recommended as part of initial triple anticoagulation therapy, patients with an indication for oral anticoagulation will also be excluded.

Finally, even though all efficacy endpoints of the present study are to be evaluated at 24 hours following PCI, it is considered in the subjects' interest to continue study treatment for a period of a month, during which period all enrolled subjects will be monitored for the safety end-point and other adverse events. This is a result of a lack of available evidence to support switching from a more potent to a less potent antiplatelet drug within 24 hours of PCI, and a paucity of studies regarding the optimal clopidogrel loading dose when switching from ticagrelor to clopidogrel.[27] Furthermore, receiving a loading dose of ticagrelor and within 24 hours a loading dose of clopidogrel combined with peri-procedural anticoagulation, may significantly increase bleeding risk.

2. STUDY OBJECTIVES

2.1 Primary objective

The primary objective of this study is to demonstrate superior efficacy of a combination of ticagrelor and RIPC in the reduction of PMI compared to a clopidogrel-RIPC combination or ticagrelor alone, by evaluating post-procedural cardiac Troponin I (cTnI) levels, defined as cTnI at 24 hours post-PCI.

2.2 Secondary objectives

Evaluation of chest pain during coronary balloon occlusion. Chest pain severity assessed with a numerical 10 point scale (0: no pain, 10: most severe discomfort ever experienced)

Evaluation of ECG evidence of ischemia during coronary balloon occlusion. ST-segment deviation as monitored during coronary balloon occlusion.

2.3 Safety objective

Safety monitoring will be performed daily during the index hospitalization. The presence of adverse events will be assessed by history, detailed physical examination and appropriate laboratory tests that will be described in the protocol accordingly.

In addition patients will be instructed to contact the study personnel in case of any emergency situation whereas an adverse event reporting system will be available throughout the study.

In detail, bleeding according to the Thrombolysis in Myocardial Infarction (TIMI) bleeding criteria will be recorded and assessed throughout study duration. According to these criteria bleeding is defined as follows:

Non-CABG Related Bleeding:

1. Major

- Any intracranial bleeding (excluding microhemorrhages <10 mm evident only on gradient-echo MRI)
- Clinically overt signs of hemorrhage associated with a drop in hemoglobin of ≥5 g/dL or a ≥15% absolute decrease in haematocrit
- Fatal bleeding (bleeding that directly results in death within 7 d)

2. Minor

- Clinically overt (including imaging), resulting in hemoglobin drop of 3 to <5 g/dL or ≥10% decrease in haematocrit
- No observed blood loss: ≥4 g/dL decrease in the haemoglobin concentration or ≥12% decrease in haematocrit

- Any overt sign of hemorrhage that meets one of the following criteria and does not meet criteria for a major or minor bleeding event, as defined above
- Requiring intervention (medical practitioner-guided medical or surgical treatment to stop or treat bleeding, including temporarily or permanently discontinuing or changing the dose of a medication or study drug)
- Leading to or prolonging hospitalization
- Prompting evaluation (leading to an unscheduled visit to a healthcare professional and diagnostic testing, either laboratory or imaging)

3. Minimal

- Any overt bleeding event that does not meet the criteria above
- Any clinically overt sign of haemorrhage (including imaging) associated with a <3 g/dL decrease in haemoglobin concentration or <9% decrease in haematocrit

Bleeding in the Setting of CABG:

- Fatal bleeding (bleeding that directly results in death)
- Perioperative intracranial bleeding
- Reoperation after closure of the sternotomy incision for the purpose of controlling bleeding
- Transfusion of ≥5 U PRBCs or whole blood within a 48-h period; cell saver transfusion will not be counted in calculations of blood products.
- Chest tube output >2 L within a 24-h period

3. STUDY PLAN AND PROCEDURES

3.1 Overall study design and flow chart

The TRIP study will be a randomized, assessor-blind, active comparator-controlled, clinical trial using a 2×2 factorial design, with 1:1 patient allocation to ticagrelor or clopidogrel and within each treatment a 1:1 allocation to RIPC or control.

According to the study plan, patients who are referred for coronary angiography or NSTE-ACS may be screened for randomization.

	Day 1/ Screening	Day 2	Day 3	Day 30 (±5days)
Informed consent form	x			
Inclusion criteria	x			
Exclusion criteria	x	x	x	

Medical history	x			x
Concomitant disease	x			x
Concomitant medication	x	x	x	x
Physical examination (vital signs)	x	x	x	x
ECG	x	x		
Local laboratory values	x	x	x	
Randomization	x	x		
Treatment initiation	x			
RIPC		x		
Coronary angiography assessment		x		
PCI		x		

Day 1 of the study is defined as the day of randomization to ticagrelor versus clopidogrel. This should be chosen by the investigator to be the day before the scheduled PCI procedure.

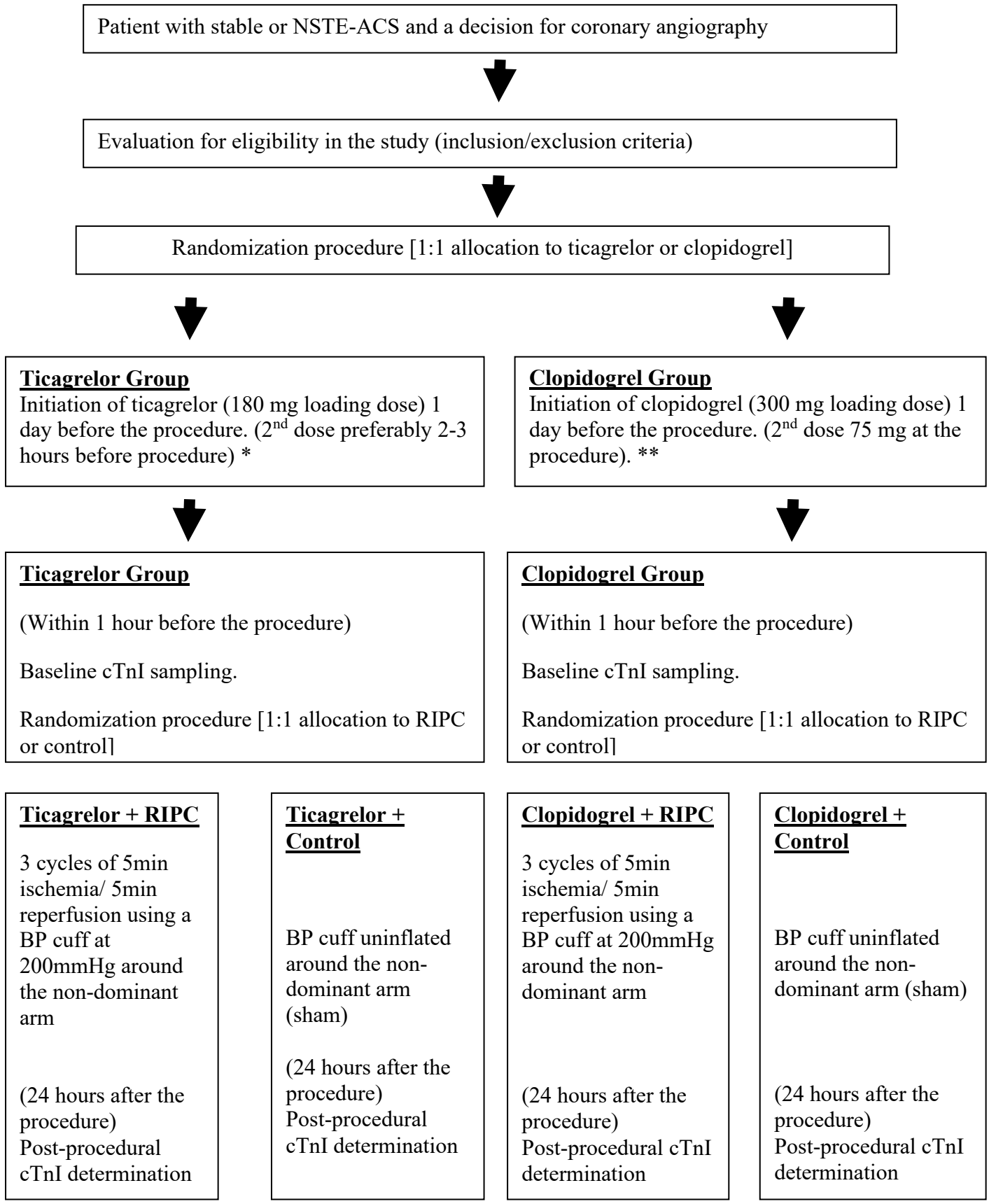
Day 2 of the study is defined as the day of randomization to RIPC or control and subsequent (within 1 hour) coronary angiography and PCI.

In patients already treated with clopidogrel for 3 to 7 days, actions performed on Day 1 and Day 2 may be combined into a single day provided that;

a. Twenty four hours have elapsed between the last dose of clopidogrel and the randomization to the ticagrelor or clopidogrel group, and

b. There is sufficient time (2-4 hours) between the randomization and subsequent intake of clopidogrel or ticagrelor and the 2nd randomization to the RIPC or control group.

If for unforeseen reasons, PCI is not conducted within the prespecified timeframe the patient should be withdrawn from the study.


Patient with stable or NSTE-ACS and a decision for coronary angiography
Evaluation for eligibility in the study (inclusion/exclusion criteria)
Randomization procedure [1:1 allocation to ticagrelor or clopidogrel]
Ticagrelor Group
Initiation of ticagrelor (180 mg loading dose) 1 day before the procedure. (2nd dose preferably 2-3 hours before procedure) *
Clopidogrel Group
Initiation of clopidogrel (300 mg loading dose) 1 day before the procedure. (2nd dose 75 mg at the procedure). **
Ticagrelor Group
(Within 1 hour before the procedure)
Baseline cTnI sampling.
Randomization procedure [1:1 allocation to RIPC or control]
Clopidogrel Group
(Within 1 hour before the procedure)
Baseline cTnI sampling.
Randomization procedure [1:1 allocation to RIPC or control]
Ticagrelor + RIPC
3 cycles of 5min ischemia/ 5min reperfusion using a BP cuff at 200mmHg around the non-dominant arm
(24 hours after the procedure)
Post-procedural cTnI determination
Ticagrelor + Control
BP cuff uninflated around the non-dominant arm (sham)
(24 hours after the procedure)
Post-procedural cTnI determination
Clopidogrel + RIPC
3 cycles of 5min ischemia/ 5min reperfusion using a BP cuff at 200mmHg around the non-dominant arm
(24 hours after the procedure)
Post-procedural cTnI determination
Clopidogrel + Control
BP cuff uninflated around the non-dominant arm (sham)
(24 hours after the procedure)
Post-procedural cTnI determination

* In case of a patient already on clopidogrel for 3-7 days, ticagrelor loading dose is administered on the day of the procedure (24 hours after the last clopidogrel dose), and no 2nd ticagrelor dose is administered. ** In case of a patient already on clopidogrel for 3-7 days, no loading dose of clopidogrel is administered, but the patient ideally receives 75mg clopidogrel 2-3 hours before the procedure.

3.2 Rationale for study design, doses and control groups

Patients allocated to RIPC will undergo a RIPC protocol within 60 minutes prior to PCI, according to previously established preconditioning protocols (CRISP stent study).[21] Antiplatelet doses in the ticagrelor and the control group are according to established practice guidelines (ESC Guidelines for Revascularization 2014).[23]

4. SUBJECT SELECTION CRITERIA

A total number of 152 patients (38 patients of each group) will be recruited from patients with stable CAD or NSTE-ACS undergoing coronary angiography, eligible for PCI

4.1 Inclusion criteria

For inclusion in the study subjects should fulfill the following criteria:

1. Provision of informed consent prior to any study specific procedures
2. Patients *(Female and male)* ≥ 18 of age
3. Patients with NSTE-ACS undergoing coronary angiography, eligible for PCI

4.2 Exclusion criteria

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

1. Women of childbearing potential
2. Severe comorbidity (estimated life expectancy <6 months)
3. Baseline cTnI before PCI that is not stable or falling or is > 5 ×99th percentile URL.
4. End-stage renal disease(eGFR<15 ml/min/1.73 m^2)
5. CRUSADE Bleeding Score >50
6. Patients with an indication for oral anticoagulation
7. On maintenance therapy with ticagrelor or those that have received clopidogrel for less than 3 days
8. Use of nicorandil or glibenclamide
9. Concomitant theophylline/aminophylline use
    I. Known contraindications to the use of ticagrelor Hypersensitivity to the active substance or to any of the excipients
    II. Active pathological bleeding
    III. History of intracranial haemorrhage
    IV. Moderate to severe hepatic impairment
    V. Co-administration of ticagrelor with strong CYP3A4 inhibitors (e.g., ketoconazole, clarithromycin, nefazodone, ritonavir, and atazanavir).

5. STUDY CONDUCT

5.1 Subject enrollment, randomization, and initiation of investigational product

According to the study protocol, patients who are referred for coronary angiography or NSTE-ACS may be screened for randomization. Subjects meeting the inclusion and none of the exclusion criteria will be evaluated and their demographic, medical history and current clinical characteristics will be recorded.

During the screening period the eligibility check and physical examination will be performed with assessment of vital signs (heart rate and blood pressure measurement). During the screening period obtained local laboratory results should be examined for exclusion criteria (eGFR, HAS-BLED score, cardiac troponin). The subject will then be informed about the study and will sign the Informed Consent Form.

Day 1 of the study is defined as the day of randomization to ticagrelor versus clopidogrel. This should be chosen by the investigator to be the day before the scheduled PCI procedure.

Day 2 of the study is defined as the day of randomization to RIPC or control and subsequent (within 1 hour) coronary angiography and PCI.

In patients already treated with clopidogrel for 3 to 7 days, actions performed on Day 1 and Day 2 may be combined into a single day provided that;

a. Twenty-four hours have elapsed between the last dose of clopidogrel and the randomization to the ticagrelor or clopidogrel group, and
b. There is sufficient time (2-4 hours) between the randomization and subsequent intake of clopidogrel or ticagrelor and the 2nd randomization to the RIPC or control group.

In these case patients are 1:1 randomized to ticagrelor or clopidogrel, and undergo the second randomization within 2-4 hours

If for unforeseen reasons, PCI is not conducted within the prespecified timeframe the patient should be withdrawn from the study.

5.1.1 Procedures for randomization

The TRIP study will be a randomized, assessor-blind, active comparator-controlled, clinical trial using a 2×2 factorial design, with 1:1 patient allocation to ticagrelor or clopidogrel and within each treatment a 1:1 allocation to RIPC or control. Patient randomization in the ticagrelor and the clopidogrel group will take place using sealed, opaque envelopes containing a computer-generated randomization scheme. Using a similar procedure, patients will be randomly assigned to RIPC or no RIPC within 1 hour before the procedure.

5.2 Procedures for handling subjects incorrectly enrolled or randomized or initiated on investigational product

Subjects who fail to meet the inclusion and exclusion criteria are defined as screening failures. The investigator will maintain a Screening Log which includes screen failures. The log will document the subject number, subject initials, demographics and the reason(s) for excluding the patient from the study. This log will be kept in the Investigator's Study File. It will be used to determine systematic bias in selection of patients for entry into the study.

Subjects who withdraw or are withdrawn from the study following randomization, before investigational product initiation should:

- Have the reason(s) for their withdrawal recorded

Subjects who withdraw or are withdrawn from the study following randomization and investigational product initiation should:

- Have the reason(s) for their withdrawal recorded
- Be asked about the presence of any AEs and if so should be followed up by regular scheduled visits, telephone contact, correspondence or home visits until satisfactory clinical resolution of AEs is achieved.
- Be seen by an investigator and all final assessments will be performed and recorded in the termination page of CRF.
- Have at least one follow-up contact for safety evaluation during the 30 days following the last dose of study treatment.
- In the event of pregnancy, the subject should be monitored until conclusion of the pregnancy and the outcome of pregnancy reported.
- Have study treatment returned (according to accountability practices)

5.3 Blinding and procedures for unblinding the study

The TRIP study will be an open label study with a blinded assessor. Accordingly, two investigators unaware of the patients' allocation will assess outcomes. In detail, each study patient will have a post-procedural cTnI measurement and a pain measurement not amenable to assessment; however the ST-segment deviation will be decided by two blinded expert investigators. In the event of discrepant assessments, final outcome will be defined by consensus between the two assessors.

5.4 Treatments

5.4.1 Identity of investigational product(s)

Investigational product	Dosage form and strength	Manufacturer
Ticagrelor	90 mg film coated tablet – BID	AstraZeneca

Clopidogrel	75 mg film coated tablet – QD	To be defined (PIs decide which clopidogrel will be used)
	300 mg film coated tablet – QD	

Ticagrelor

Chemical name (IUPAC): (1S,2S,3R,5S)-3-[7-{[(1R,2S)-2-(3,4Difluorophenyl)cyclopropyl]amino}-5-(propylthio)3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl]-5-(2hydroxyethoxy)cyclopentane-1,2-diol Laboratory code: AZD6140 (previously AR-C126532XX)

Chemical structure

Ticagrelor has 6 asymmetric carbon atoms, all of which are prepared by synthesis. All stereocentres are introduced by stereo-selective chemistry early in the synthetic pathway where correct configuration is established.

Molecular formula: C23H28F2N6O4S

Relative molecular mass: 522.57

Partition coefficient, log P (octanol/water): >4.0

General properties

Ticagrelor is a crystalline powder with an aqueous solubility of approximately 10 μg/mL at room temperature. During routine manufacture solid ticagrelor is only isolated as a single polymorph, which has been used in all toxicological and clinical studies to date.

Drug product

Presentation

Ticagrelor will be provided as Brilique tablets. The drug product is a conventional immediate release (IR) tablet for oral use. One tablet strength will be available:90 mg tablets - presented as round, biconvex, yellow, film coated tablets

Composition

The composition of the ticagrelor tablet core is ticagrelor, mannitol, dibasic calcium phosphate, sodium starch glycolate, hydroxypropyl cellulose, and magnesium stearate. The composition of the tablet coating is: hydroxypropyl methylcellulose, titanium dioxide, talc, polyethylene glycol 400, and iron oxide yellow (45 mg and 90 mg tablet strengths); and hydroxypropyl methylcellulose, titanium dioxide, talc and polyethylene glycol 400 (60 mg tablet strength).

Clopidogrel

Clopidogrel is a thienopyridine class inhibitor of $P2Y_{12}$ ADP platelet receptors. Chemically it is methyl (+)-(S)-α-(2-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H) acetate sulfate (1:1). The empirical formula of clopidogrel bisulfate is C16H16ClNO2S•H2SO4 and its molecular weight is 419.9.

Clopidogrel will be provided as Plavix 75mg tablets or equivalent according to local availability.

5.4.2 Doses and treatment regimens

For the ticagrelor group: loading dose 180 mg ticagrelor (2×90mg tablets). Thereafter, 90mg (1 tablet) b.i.d. At the end of the study period, treatment should be continued according to the prescription of the investigator and the attending physician.

For the clopidogrel group: Patients not on clopidogrel - loading dose 300 mg clopidogrel (4×75mg tablets). At the time of PCI an additional 75 mg dose. Thereafter, 75mg o.d. Patients already on clopidogrel – continuation of maintenance dose. At the end of the study period, treatment should be continued according to the prescription of the investigator and the attending physician.

5.4.3 Additional study drug

Ticagrelor or clopidogrel will be given as part of dual antiplatelet treatment in combination with aspirin at an initial loading dose of 150-300mg (according to primary investigator preference) and 100mg maintenance dose thereafter.

5.4.4 Labeling

Packaging and labelling procedures for the study medication will be followed according to the local regulatory requirements and regulations for interventional studies.

5.4.5 Storage

The product should be stored in the pack provided and used according to the instructions on the label.

5.5 Concomitant and post-study treatment(s)

All medications taken by the patient during the treatment period in addition to the study medication, are termed concomitant medication. All concomitant medication taken during the treatment period of the study will be documented on the case report form (trade name, start and stop date and daily dose). Concomitant medication must also be recorded in the patient's records. All prior medications administered within 1 week of enrolment into the study must be documented on the case report form.

5.6 Treatment compliance

Inpatient administration will be documented (date, time, dose, and signature of dispensing person). A patient diary will be used to document outpatient administration. Drug accountability of the unused study medication will be performed. The treatment compliance should be between 80% and 120%.

5.6.1 Accountability

Each patient will be dispensed sufficient medication for one month of therapy. Upon dispensation, the investigator must write the following in the Investigational Product Dispensing Log: subject ID and initials and date dispensed, the total dose given

weekly/monthly and frequency of dosage, total bottles dispensed, batch number and expiry date of product.

The investigator or designee must maintain current and accurate record of the receipt, inventory and dispensing, including shipping invoices, of all study supplies. The Investigational Product Accountability Log must include:

- Date received
- Delivery order (D.O.#) reference number and amount received and placed in storage
- Name of study medication and dosage
- Amount currently in storage area
- Label ID number or batch number/Lot number
- Name and initial of person responsible for each investigational product inventory entry/movement
- Amount dispensed to and returned by each subject, including unique subject identifiers
- Amount transferred to another area for dispensing or storage
- Non-study disposition (eg. Lost, wasted, broken)
- Amount returned to sponsor
- Amount destroyed at study site ID number or batch number/Lot number

5.7 Discontinuation of investigational product

Patients who require discontinuation of ticagrelor are at increased risk for cardiac events. Premature discontinuation of treatment should be avoided. If ticagrelor must be temporarily stopped due to an adverse event(s), it should be re-initiated as soon as possible when the benefits outweigh the risks of the adverse event or when the adverse event has come to resolution.

5.7.1 Procedures for discontinuation of a subject from investigational product

Patients who require discontinuation of ticagrelor should receive dual antiplatelet treatment for a period, which is defined by disease presentation (stable CAD or NSTE-ACS) and type of stent (or stents) used during PCI. The investigator and the subjects' physicians should base the decision for ticagrelor discontinuation, switch to clopidogrel, and required loading doses on relevant clinical practice guidelines and established local clinical practice.[23]

Subjects who discontinue the investigational product should:

- Have the reason(s) for discontinuation recorded
- Be asked about the presence of any AEs and if so should be followed up by regular scheduled visits, telephone contact, correspondence or home visits until satisfactory clinical resolution of AEs is achieved.

- Be seen by an investigator and all final assessments will be performed and recorded in the termination page of CRF.
- Have at least one follow-up contact for safety evaluation during the 30 days following the last dose of study treatment.
- In the event of pregnancy, the subject should be monitored until conclusion of the pregnancy and the outcome of pregnancy reported.
- Have study treatment returned (following previously described accountability practices)

5.8 Withdrawal from study

Subjects have the right to withdraw from the study at any time for any reason, including personal reasons. The Investigator also has the right to withdraw subjects from the study in the event of:

- An adverse event which is considered intolerable by the subject or the Investigator and to interfere with the continuation of the survey or the subject evaluation.
- An abnormal laboratory test result which is considered clinically significant by the Investigator and possibly interfering with the continuation of the survey or the subject evaluation.
- The development of an exclusion criterion.

It is understood by all concerned Investigators that an excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawals of subjects should be avoided.

However, should a subject definitely decide to withdraw, all efforts will be made by the Investigator to complete and report the final observations as thoroughly as possible. A complete final evaluation at the time of the withdrawal will be performed with an explanation of the exact reason why the subject is withdrawing from the study in the “Study Completion Summary” of the CRF.

6. COLLECTION OF STUDY VARIABLES

6.1 Recording of data

Data collection and recording will be performed through paper case report forms (pCRFs) that will be provided by the Sponsor to the participating site for each subject separately. The use of a waterproof ballpoint black pen is recommended for the record of data on the pCRF. The investigator should record all relevant data in the appropriate CRF and should ensure the accuracy, completeness, legibility and timeliness of the data reported to the sponsor in the CRFs and in all required reports. Accurate and reliable data collection will be assured by verification and cross-check of 100% of the CRFs against the investigator's records (source document verification).

6.2 Data collection at enrolment and follow-up

Screening (in most cases expected to be the same day with first randomization-Day1)

During this period the investigator will evaluate and record

- Indication for coronary angiography or PCI (stable CAD, NSTE-ACS)
- Demographics (age, gender, height, weight, body mass index)
- History of risk factors for cardiovascular disease (Diabetes mellitus, dyslipidemia, hypertension, smoking)
- Medical history including history of previous MI, previous PCI, previous CABG, New York Heart Association Class, Canadian Cardiology Society Angina Class
- Prior and concomitant medication
- Physical examination with vital signs (heart rate, blood pressure)
- Electrocardiogram
- Local laboratory values (hemoglobin, complete blood count including platelet count, serum creatinine, AST, ALT, INR, cardiac Troponin I)

Day 2 - Pre-procedural assessment

- Physical examination with vital signs (heart rate, blood pressure). At this time signs and symptoms of bleeding should be specifically examined.
- Local laboratory values (hemoglobin, complete blood count including platelet count, serum creatinine, AST, ALT, INR, cardiac Troponin I)

In the case of patients already on clopidogrel there will be one combined assessment for screening and pre-procedurally.

Day 2 - Preconditioning

- The timing of the last cycle of RIPC in relation to subsequent PCI should be specifically recorded

Day 2 - Peri-procedural

- Angiographic parameters (number of diseased vessels, target vessel, jeopardy score, rentrop score, American heart association lesion type, stenosis severity, acute gain, timi flow score)
- Procedural parameters (access site, stent length, type of stent used, screen time, radiation dose, contrast dose, number of predilations, predilation time, number of postdilations, postdilation time)
- Complications (jailed side branch, dissection)
- ST-segment deviation during balloon occlusion will be monitored as a secondary efficacy variable. It will be defined as the absolute value of ST-segment deviation at 60-80ms after the J-point in mm at the beginning of coronary angiography minus ST-segment deviation at 60-80ms after the J-point in mm during balloon occlusion.

Day 2 - Post-procedural

- Physical examination with vital signs (heart rate, blood pressure). At this time signs and symptoms of access site bleeding should be specifically examined.
- Electrocardiogram
- Chest pain during PCI will be assessed during the post-PCI clinical examination of the subject by the investigator or by an appropriately qualified person to whom the investigator has delegated this duty. A numerical 10 point scale will be used (0: no pain, 10: most severe discomfort ever experienced)

Day 3

- Physical examination with vital signs (heart rate, blood pressure). At this time signs and symptoms of access site bleeding should be specifically examined.
- Local laboratory values, i.e. hemoglobin, complete blood count including platelet count, serum creatinine, AST, ALT, INR, and Cardiac troponin I at 24 hours post-PCI

Day 4-day 29 (Follow-up)

- Physical examination with vital signs (heart rate, blood pressure), while admitted in the hospital. After discharge the patiens will be instructed to inform the investigator for potential adverse events.

Day 30 Follow-up visit

- Physical examination with vital signs (heart rate, blood pressure). Signs and symptoms of bleeding should be specifically examined.

6.3 Efficacy

6.3.1 Efficacy variables

The primary efficacy variable, cardiac troponin I at 24 hours post-PCI will be determined according to local standard clinical practice. In detail, cTnI will be analyzed with an automated immunoassay (Bayer ADVIA IMS Troponin-I Ultra method, Bayer, Berlin, Germany). The 99th percentile of the cTnI level in a reference population (upper reference, limit, URL) of healthy volunteers was below the lower limit of detection of 0.04 ng/mL. The coefficient of variation (CV) of the assay was <10%, complying with the recommendations of the European Society of Cardiology (ESC)/ American College of Cardiology (ACC)/ American Heart Association (AHA) for optimal precision. The analytical range was 0.01 to 50ng/mL, with an assay sensitivity of 0.006 ng/mL.

Chest pain during PCI is a secondary efficacy variable. It will be assessed during the post-PCI clinical examination of the subject by the investigator or by an appropriately qualified person to whom the investigator has delegated this duty. An analog 10 point scale will be used (0: no pain, 10: most severe discomfort ever experienced).

ST-segment deviation during balloon occlusion will be monitored as a secondary efficacy variable. It will be defined as the absolute value of ST-segment deviation at 60-80ms after the J-point in mm at the beginning of coronary angiography minus ST-segment deviation at 60-80ms after the J-point in mm during balloon occlusion.

6.4 Safety

The Principal Investigator is responsible for ensuring that all staff involved in the study is familiar with the content of this section.

6.4.1 Definition of adverse events

An adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical study subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (eg, tachycardia, enlarged liver, or an abnormal laboratory finding), symptom (eg, nausea, chest pain), or disease temporally associated with the use of a study drug, whether or not considered related to the study drug. In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

The term AE is used to include both serious and non-serious AEs.

6.4.2 Definitions of serious adverse event

A serious adverse event is an AE occurring during any study phase (ie, run-in, treatment, washout, follow-up), that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above.

The causality of SAEs (their relationship to all study treatment/procedures) will be assessed by the investigator(s) and communicated to AstraZeneca.

6.4.3 Recording of adverse events

Time period for collection of adverse events

Adverse Events will be collected from the time of signature of informed consent throughout the study treatment period (including the 30-day follow-up period post-PCI), up to 30 days after randomization.

SAEs will be recorded from the time of informed consent and up to 30 days after randomization.

Follow-up of unresolved adverse events

Any AEs that are unresolved at the subject's last AE assessment in the study are followed up by the Investigator for as long as medically indicated, but without further recording in the CRF. Sponsor or its designee retains the right to request additional information for any subject with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

Variables

The following variables will be collect for each AE;

1. AE (verbatim)
2. The date and time when the AE started and stopped
3. Severity

   Note: the following severity rating scale will be used:

   - *mild (awareness of sign or symptom, but easily tolerated for outpatients, and awareness of sign or symptom, but easily tolerated for in-patients)*
   - *moderate (discomfort sufficient to cause interference with normal activities for outpatients, and disturbing but still tolerable for in-patients)*
   - *severe (incapacitating, with inability to perform normal activities for outpatients, and intolerable for outpatients)*
4. Whether the AE is serious or not
5. Investigator causality rating against the Investigational Product (not related, possible, or probable)
6. Action taken with regard to investigational product(s)
7. AE caused subject's withdrawal from study *(*yes or no*)*
8. Outcome.

In addition, the following variables will be collected for SAEs:

1. Date AE met criteria for serious AE
2. Date Investigator became aware of serious AE
3. AE is serious due to
4. Date of hospitalisation
5. Date of discharge
6. Probable cause of death
7. Date of death
8. Autopsy performed
9. Causality assessment in relation to Study procedure(s)
10. Causality assessment in relation to Other medication
11. Description of AE.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 6.4.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not a SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be a SAE.

The Sponsor or its designee will classify events using Medical Dictionary for Regulatory Activities (MedDRA).

Causality collection

The Investigator will assess causal relationship between Investigational Product and each Adverse Event, and answer 'yes' or 'no' to the question 'Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?'.

For SAEs causal relationship will also be assessed for other medication and study procedures. Note that for SAEs that could be associated with any study procedure the causal relationship is implied as 'yes'.

Adverse Events based on signs and symptoms

All AEs spontaneously reported by the subject or reported in response to the open question from the study personnel: *<<'Have you had any health problems since you were last asked?>> or* revealed by observation will be collected and recorded in the CRF. When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

Adverse Events based on examinations and tests

The results from protocol mandated laboratory tests and vital signs will be summarized in the clinical study report. Deterioration as compared to baseline in protocol-mandated laboratory values and vital signs should therefore only be reported as AEs if they fulfil any of the SAE criteria or are the reason for discontinuation of treatment with the investigational product.

If deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. Wherever possible the reporting Investigator uses the clinical, rather than the laboratory term (eg, anaemia versus low haemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AE(s).

Deterioration of a laboratory value, which is unequivocally due to disease progression, should not be reported as an AE/SAE.

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

Disease progression or worsening of pre-existing conditions

Disease progression can be considered as a worsening of a subject's condition attributable to the disease (i.e., STEMI) for which the investigational product is being studied.

It may be an increase in the severity of the disease under study and/or increases in the symptoms of the disease. The development of ischaemia and symptoms of coronary artery disease, artery disorders, cardiac failure, pericardium disorders, arrhythmias and other MI-related cardiac symptoms/disorders should be considered as disease progression and not an AE.

Events, which are unequivocally due to disease progression, should not be reported as an AE during the study.

Worsening of the disease under study will be recorded as an AE only if one of the following criteria is met:

- *Worsening of disease meets the criteria for an SAE.*
- *Action is taken with investigational drug, i.e. dose is reduced or treatment discontinued or increased.*
- *Concomitant medication is added or changed.*
- *The investigator believes a subject has shown a clear, unexpected deterioration from baseline symptoms.*

The same criteria as above apply for the recording of AEs that result from worsening of other pre-existing diseases/conditions. In particular, the following will not qualify for recording as an AE:

- *Pre-existing conditions present at baseline, which remain unchanged during the study.*
- *Expected fluctuations or expected deterioration of a pre-existing disease/condition.*

Reporting of serious adverse events

The Sponsor and Principal Investigator are responsible for meeting all local regulatory requirements and obligations.

All SAEs have to be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). All SAEs will be recorded on the SAE page as well as on the relevant AE module of the pCRF.

If any SAE occurs in the course of the study, then Investigator or other site personnel should inform the Sponsor or its designee immediately, or **no later than 24 hours** from awareness of the event.

The Sponsor or its designee will work with the Investigator to ensure that all the necessary information is provided to the Safety database, as appropriate.

For fatal or life-threatening adverse events where important or relevant information is missing, active follow-up is undertaken immediately. Investigator or other site personnel should inform

the Sponsor or its designee of any follow-up information on a previously reported SAE immediately, or **no later than 24 hours** from awareness of the event.

Investigator or other site personnel send relevant SAE form by fax or e-mail (scanned copies) to the Sponsor or its designee.

The reference documents for definition of expectedness of an AE is the investigational products' latest approved prescribing information (SmPCs).

The Sponsor or its designee is responsible for reporting to the National Ethics Committee and the European Medicines Agency (EMA), of the Serious Unexpected Suspected Adverse Drug Reactions (SUSARs) occurring in the study, including SUSARs associated with active comparator(s).

SUSARs are forwarded electronically by the Sponsor or its designee to Receiver ID "EVCTMPROD" in the production environment of Eudravigilance.

The reporting timelines are the following:

- Fatal or life-threatening SUSARs: within 7 days of Sponsor's (or its designee) date of awareness
- All other SUSARs within 15 days of Sponsor's (or its designee) date of awareness.

Sponsor's or its designee contact details are the following:

Name of Responsible Person: Theodoros Zografos

Address: Artemidos 8,Vari, Athens, Greece
Tel.: +302108976005
Mob.: +306956161001
Fax: +302108976005
E-mail: theodoroszografos@gmail.com

The Sponsor or its designee or the Principal Investigator is required, to notify AstraZeneca of all SUSARs, as soon as possible and at least at the same time that the reports are sent to the Eudravigilance. Reports should be sent to AstraZeneca on a CIOMS form to the following e-mail address: <AEMailboxClinicalTrialTCS@astrazeneca.com .

In addition, the Principal Investigator must provide AstraZeneca with the same report of all other SAEs that did not qualify for expedited reporting at the same e-mail address and on an expedited basis. These reports must be submitted to AstraZeneca quarterly.

The Sponsor or its designee when providing local periodic study reports to the local Regulatory Authority and any IRB/IEC updates shall at the same time forward a copy of such documentation to AstraZeneca.

The Principal Investigator is responsible for reconciling the clinical data of the ISS on an ongoing basis to ensure that all new and updated information regarding SAEs has been sent to AstraZeneca either as individual expedited SUSARs or as part of a line listing.

The Sponsor shall ensure that safety information provided by AstraZeneca is promptly distributed to local Regulatory Authority, National Ethics Committee and to any other Investigators participating in the ISS, in accordance with Applicable Laws and Regulations.

6.4.4 Laboratory safety assessment

Daily tests for hemoglobin, complete blood count including platelet count, serum creatinine, AST, ALT, INR, cardiac Troponin I on Day 1, Day 2, and Day 3.

For blood volume see Section 7.1.

6.4.5 Physical examination

Daily physical examination will be performed on Day 1, Day2, Day 3 and until discharge. Final examination on day 30 (follow-up visit)

6.4.6 ECG

6.4.6.1 Resting 12-lead ECG

On Day 1 and Day 2. Furthermore, whenever it is considered appropriate according to symptoms and physical findings. Particular inspection for bradycardia, conduction disturbances, ventricular pauses.

6.4.6.2 Real time monitoring

Will be only applied during the coronary angiography and the PCI procedure, unless patient status dictates further monitoring or CCU admission, depending on the investicator's judgement.

6.4.7 Vital signs

Daily physical examination with vital signs (heart rate, blood pressure) will be performed on Day 1, Day2, Day 3 and until discharge. Final examination with vital signs (heart rate, blood pressure) on day 30 (follow-up visit)

6.4.8 Other safety assessments

During physical examination after antiplatelet initiation and after PCI, physical examination will be specifically guided to include assessment of symptoms and signs indicative of general or access site bleeding

6.5 Patient reported outcomes (PRO)

Chest pain severity during balloon occlusion at the time of PCI will be reported by the patients during the post-PCI assessment according a numerical 10 point scale (0: no pain, 10: most severe discomfort ever experienced)

7. BIOLOGICAL SAMPLING PROCEDURES

All laboratory parameters will be determined in a local laboratory according to standard clinical practice. No additional tests will be performed on the patients enrolled in the study,

apart from the ones performed as standard clinical practice on patients with stable CAD or NSTE-ACS undergoing coronary angiography and PCI

7.1 Volume of blood

According to local clinical practice, approximately 8 ml of blood will be drawn each time for laboratory testing

7.2 Handling, storage and destruction of biological samples

All biological samples will be handled according to local standard clinical practice. No storage of biological samples will be performed.

7.3 Labeling and shipment of biohazard samples

Not applicable.

7.4 Chain of custody of biological samples

Not applicable.

7.5 Withdrawal of informed consent for donated biological samples

Not applicable

8. ETHICAL AND REGULATORY REQUIREMENTS

8.1 Ethical conduct of the study

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the Sponsor and investigator abide by Good Clinical Practice Guidelines and under the guiding principles detailed in the declaration of Helsinki. The study will also be carried out in keeping with applicable local laws and regulations. This may include an inspection by the Sponsor representatives and/or Regulatory Authority representatives at any time. The investigator must agree to the inspection of study-related records by the Regulatory Authority/Sponsor representatives, and must allow direct access to source documents to the Regulatory Authority/ Sponsor representatives.

Modifications to the study protocol will not be implemented by either the Sponsor or the investigator without agreement by both parties. However, the investigator may implement a deviation form, or a change of, the protocol to eliminate an immediate hazard to the trial subjects without prior EC/IRB/Sponsor approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it and if appropriate the proposed protocol amendment should be submitted to the EC/IRB/Sponsor. Any deviations from the protocol must be fully explained and documented by the investigator.

8.2 Ethics and regulatory review

Documented approval from appropriate EC(s)/IRBs will be obtained for all participating centers prior to study start, according to GCP, local laws, regulations, and organizations. When necessary, an extension, amendment, or renewal of the Ethics Committee approval must be obtained and also forwarded to the Sponsor. The Ethics Committees must supply to the Sponsor, upon request, a list of the Ethics Committee members involved in the vote and a

statement to confirm that the Ethics Committee is organized and operates according to GCP and applicable laws and regulations.

Regulatory Authority approvals/ authorizations/ notifications, where required, must be in place and fully documented prior to study start

8.3 Informed consent

A core information and Informed Consent Form will be provided. Prior to the beginning of the study, the investigator must have the ECs/IRB written approval/ favorable opinion of the written Informed Consent Form (ICF) and any other written information to be provided to subjects. The written approval of the EC/ IRB together with the approved subject information/ ICFs together with the approved subject information/ICFs must be filed in the study files.

Written informed consent must be obtained before any study specific procedure taked place. Participation in the study and date of informed consent given by the subject should be documented appropriately in the subject's files.

8.4 Changes to the protocol and informed consent form

No protocol, informed consent process, or informed consent document may be modified without prior approval from the IRB unless it is necessary to eliminate an apparent and immediate hazard to one or more of the participants. Such modifications consist of revisions of or amendments to existing study documents. Any planned modification must be submitted to the IRB for review and approval before being implemented or used with participants

8.5 Audits and inspections

Monitoring and auditing procedures defined/ agreed by the Sponsor will be followed, in order to comply with Good Clinical Practice (GCP) guidelines. The center will be visited at regular intervals by a monitor to ensure compliance with the study protocol, GCP and legal aspects. This will include on-site checking of the case report forms (CRF) for completeness and clarity, cross-checking with source documents, and clarification of administrative matters.

9. STUDY MANAGEMENT

9.1 Training of study site personnel

Investigators involved in this study must not enroll any patient prior to completion of a formal meeting conducted by the Clinical Research Associate designated by the Sponsor. This meeting will include an inventory of study supplies, a detailed review of the protocol and CRF, training on study procedures and other procedures required of GCP. Investigators who are not GCP certified will undergo GCP training during the study.

9.2 Monitoring of the study

9.2.1 Source data

The investigator must maintain adequate and accurate records to document the conduct of the study and substantiate the study data. These documents comprise Essential Documents Source Documents. Source documents are original hospital records, clinical charts, subject screening checklist, original laboratory reports, pharmacy dispensing records, recorded data from

automated instruments, transcriptions certified after verification as being accurate, electronic media, subjects' files, and records kept at the pharmacy, at the laboratories and at medicolegal departments involved in the study.

The investigator must maintain source documents for each patient in the study. All information on CRFs must be traceable to these source documents.

9.3 Study timetable and end of study

Contract execution to IRB/EC Approval	6 months
IRB/EC approval to first subject In	1 month
First subject in to 50% enrollment	6 months
50% enrollment to last subject in	6 months
Last subject in to last subject last visit	1 month
Last subject last visit to completion of final study report	3 months
Last subject last visit to abstract submission	4 months
Last subject last visit to manuscript submission	6 months

10. DATA MANAGEMENT

Data collection and recording will be performed through paper case report forms (pCRFs) that will be provided by the Sponsor to the participating site for each subject separately. The use of a waterproof ballpoint black pen is recommended for the record of data on the pCRF.

The Principal Investigator must review the CRFs for completeness and accuracy and must sign and date the appropriate CRF page. All CRFs must be filled out legibly.

Corrections to the data on the CRFs must only be made by drawing a straight line through the incorrect data and by writing the correct value/data next to the information that has been crossed out. Corrections must only be performed by the Principal Investigator or his qualified designees. Each correction must be initialed and dated by the Principal Investigator or his/her authorized staff.

Erasures of incorrect data with the use of correction fluid or tape are not allowed.

The monitor of the study will review and check all CRFs, for accuracy (including source data verification) and completeness and subsequently the Investigator shall correct all forms with

missing information and/or incorrect entries. Every documentation inserted on the CRF, corrections and other changes shall be performed exclusively by the responsible Investigator or the authorized by the Investigator person.

With the exception of the evident errors, all corrections must be accompanied with explanatory comments.

Clinical data will be managed by appropriate Data-management staff, who will be responsible for constructing a database containing all previously described data for the purpose of data analysis. The data-management staff will perform checks of data consistency and completeness. The data review will permit the generation of queries for the clarification of unclear/ missing/ inconsistent data. The errors found will be assessed by the Data Manager of the study and investigators will be involved in resolving them. All changes to the database will be recorded in an audit trail file. If a change is necessary once the investigator has no further access to the database, a query will be sent to the investigator for confirmation of the change. The investigator's signature is requested to demonstrate approval of the change that was made.

The investigator will be responsible for retaining all records pertaining to the study as specified in the appropriate contract.

At the end of the study, a copy of all data-sets will be provided to the Sponsor on electronic support. A study report will be written at the end of the study. Information on the study results will be given to the Regulatory Authorities, within required times, as indicated by local regulations.

11. EVALUATION AND CALCULATION OF VARIABLES

11.1 Calculation or derivation of efficacy variable(s)

The primary efficacy variable, cardiac troponin I at 24 hours post-PCI will be determined according to local standard clinical practice. In detail, cTnI will be analyzed with an automated immunoassay (Bayer ADVIA IMS Troponin-I Ultra method, Bayer, Berlin, Germany). The 99th percentile of the cTnI level in a reference population (upper reference, limit, URL) of healthy volunteers was below the lower limit of detection of 0.04 ng/mL. The coefficient of variation (CV) of the assay was <10%, complying with the recommendations of the European Society of Cardiology (ESC)/ American College of Cardiology (ACC)/ American Heart Association (AHA) for optimal precision. The analytical range was 0.01 to 50ng/mL, with an assay sensitivity of 0.006 ng/mL.

Chest pain during PCI is a secondary efficacy variable. It will be assessed during the post-PCI clinical examination of the subject by the investigator or by an appropriately qualified person to whom the investigator has delegated this duty. A numerical 10 point scale will be used (0: no pain, 10: most severe discomfort ever experienced).

ST-segment deviation during balloon occlusion will be monitored as a secondary efficacy variable. It will be defined as the absolute value of ST-segment deviation at 60-80ms after the J-point in mm at the beginning of coronary angiography minus ST-segment deviation at 60-80ms after the J-point in mm during balloon occlusion.

11.2 Calculation or derivation of safety variable(s)

The frequency of bleeding according the TIMI criteria will be recorded.

12. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION

12.1 Description of analysis sets

All variables will be analyzed descriptively with appropriate statistical methods: categorical variables by frequency tables and continuous variables by sample statistics (i.e. mean, standard deviation, minimum, median, quartiles, and maximum).

Demographic variables and baseline characteristics will be summarized by treatment group for 2 analysis populations (i.e. valid for safety analysis and valid for per protocol analysis of primary efficacy endpoint). Medical history findings and adverse events will be coded by Medical Dictionary for Regulatory Activities (MedDRA) codes and medications by Anatomic Therapeutic Chemical Classification System (ATC) codes (World Health Organization-Drug Dictionary [WHO-DD]).

12.1.1 Efficacy analysis set

A randomized subject will be considered valid for per protocol efficacy analysis if the subject:

- has received study medication according to the study protocol
- has been subjected to a RIPC or a sham procedure according to the study protocol
- has undergone PCI within one hour after RIPC or sham procedure
- has provided a valid blood sample for cTnI determination at 24 hours post-pCI

12.1.2 Safety analysis set

A randomized subject will be considered valid for safety analysis if at least 1 dose of study medication has been administered.

12.2 Methods of statistical analyses

The efficacy analysis will be performed in the population of subjects valid for safety analysis.

The statistical assessment of the primary outcome measure (post-procedural cTnI) will be performed using ANCOVA (analysis of covariance), with post-procedural cTnI as a dependent variable, -ticagrelor/clopidogrel treatment and –RIPC/no RIPC as fixed factors and pre-procedural cTnI as a covariate. If not mentioned otherwise, all statistical tests will be performed tow-sided with a type I error rate of 5% and 95% confidence intervals (two-sided) will be given.

The statistical assessment of the secondary outcome measures (pain score, and ST-segment deviation) will be performed using ANOVA (analysis of variance). The ANOVA tests the null hypothesis that samples in two or more groups are drawn from populations with the same mean values. If the ANOVA test is statistically significant, pairwise comparison of subgroups will be performed with the Tukey-Kramer test. If not mentioned otherwise, all statistical tests will be performed tow-sided with a type I error rate of 5% and 95% confidence intervals (two-sided) will be given.

The safety analysis will be performed in the population of subjects valid for safety analysis.

The incidence of the composite of major and non-major bleeding according to the TIMI criteria and the incidence of major bleeding will be tabulated and further analyzed using Fisher's exact test. Fisher's exact test is a significance test for a 2x2 table. This test evaluates all distribution probabilities for a 2 x 2 table and produces an exact probability for a given set of observed frequencies. The null hypothesis is that the row and column variables are unrelated, or that there is no difference in the respective proportions. If not mentioned otherwise, all statistical tests will be performed two-sided with a type I error rate of 5% and 95% confidence intervals (two-sided) will be given.

12.2.1 Interim analyses

No interim analysis will be scheduled

12.3 Determination of sample size

According to our previous research[19] and other available studies on the subject[20] we have assumed post-procedural cTnI to be approximately 0.10ng/mL in the RIPC group (0.08ng/mL for the ticagrelor group and 0.12 for the clopidogrel group) and approximately 0.19ng/mL for the control group (0.14ng/mL for the ticagrelor group and 0.24 for the clopidogrel group), with a standard deviation of 0.20 for both groups. According to our previous experience the correlation coefficient between preprocedural and postprocedural cTnI is approximately 0.7. Based on previously published methods,[28,29] we have estimated a sample size of 152 patients (38 patients for each group) to achieve a statistical power of 80% with a=0.05, incorporating a dropout rate of 15% (pci failure, revoked consent, etc.)

Statistical analyses have been performed using IBM SPSS Statistics v21, (IBM Corporation), and Power Analysis v1.4 (A&X Analytics).

13. IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

13.1 Overdose

There is currently no known antidote to reverse the effects of ticagrelor, and ticagrelor is not expected to be dialyzable. Treatment of overdose should follow local standard medical practice.

The expected effect of excessive ticagrelor dosing is prolonged duration of bleeding risk associated with platelet inhibition. If bleeding occurs, appropriate supportive measures should be taken.

Ticagrelor is well tolerated in single doses up to 900 mg. Gastrointestinal toxicity was dose-limiting in a single ascending dose study. Other clinically meaningful adverse effects which may occur with overdose include dyspnoea and ventricular pauses.

In the event of overdose, observe for these potential adverse effects and consider electrocardiogram monitoring.

13.2 Pregnancy

All outcomes of pregnancy should be reported to AstraZeneca. The Investigator should substitute ticagrelor with an acceptable alternative, according to local practice, if dual antiplatelet treatment is considered appropriate.

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