Clinical Study Protocol

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Antiplatelet therapy effect on platelet extracellular vesicles in acute myocardial infarction (AFFECT EV)

Sponsor: Medical University of Warsaw

The following Amendment(s) and Administrative Changes have been made to this protocol since the date of preparation:

Amendment No.	Date of Amendment	Local Amendment No:	Date of Local Amendment

Administrative Change No.	Date of Administrative Change	Local Administrative Change No.	Date of Local Administrative Change

PROTOCOL SYNOPSIS

Antiplatelet therapy effect on platelet extracellular vesicles in acute myocardial infarction (AFFECT EV)

Principal Investigator

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Study site(s) and number of subjects planned

1st Chair and Department of Cardiology, Medical University of Warsaw, 60 subjects

Study period		Phase of development
Estimated date of first subject enrolled	28.12.2016	4
Estimated date of last subject completed	28.12.2017	4

Study design

This is a prospective, single-centre, randomised, open-label, parallel group design, phase 4, proof-of-concept study conducted at the 1st Chair and Department of Cardiology, Medical University of Warsaw, Poland. The analytical part will be conducted in the Vesicle Observation Centre, Academic Medical Centre, University of Amsterdam, The Netherlands. The duration of this study is expected to be 12 months.

Objectives

Primary Objective:	Outcome Measure:

To compare the effects of ticagrelor and clopidogrel on the concentration of PEVs in patients with acute myocardial infarction.	Concentration of PEV exposing P-selectin and PS (flow cytometry).

Secondary Objective:	**Outcome Measure :**
To compare the effects of ticagrelor and clopidogrel on the concentration of EVs exposing fibrinogen, exposing phosphatidylserine, EVs from endothelial cells, EVs from leukocytes.	Concentration of EV exposing fibrinogen, exposing phosphatidylserine, EVs from endothelial cells, EVs from leukocytes.

Safety Objective:	**Outcome Measure :**
To evaluate the safety and tolerability of ticagrelor in relation to clopidogrel. To be reported descriptively.	- Vital signs - Abnormal blood tests values (haematology and clinical chemistry) - Abnormal values in urinalysis - Abnormalities in 12-lead ECG - AEs/ SAEs

Target subject population

Subjects with the first ST-elevation acute myocardial infarction (STEMI) or non-STEMI (NSTEMI) who underwent percutaneous coronary intervention (PCI) with stent implantation and received a loading dose of clopidogrel (600 mg) prior to PCI.

Duration of treatment

6 months

Investigational product, dosage and mode of administration

All drugs, including the investigational product ticagrelor, will be administered orally. All subjects will be administered a loading dose of clopidogrel (600 mg) prior to PCI. Subjects randomised to ticagrelor will receive a loading dose of ticagrelor (180 mg), followed by a maintenance dose (90 mg twice daily). Subjects randomised to clopidogrel will continue the treatment with a maintenance dose of clopidogrel (75 mg once daily). According to the guidelines of European Society of Cardiology, double antiplatelet therapy with acetylsalicylic acid (ASA) and a P2Y12 receptor antagonist will be continued for 12 months. The study treatment will last for 6 months until the last visit at the end of the treatment (LSLV). After termination of the study patients will be prescribed double antiplatelet therapy for the remaining 6 months. Ticagrelor will be recommended over clopidogrel. Subjects who choose to switch to clopidogrel due to financial constraints will be prescribed clopidogrel.

Statistical methods

Categorical variables will be presented as number (%); continuous variables will be presented as mean and standard deviation (SD) or median with inter-quartile range. Shapiro–Wilk test will be used to test for non-Gaussian distribution of continuous variables. Student's two-sided t-test or ANOVA-repeated measures will be used for normally distributed values. Mann–Whitney U test or Friedman test will be used for variables without normal distribution. A p-value below 0.05 will be considered significant.

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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
AE	Adverse event
CRF	Case Report Form (electronic/paper)
CSA	Clinical Study Agreement
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Event
DAE	Discontinuation of Investigational Product due to Adverse Event
DNA	Deoxyribonucleic acid
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation
IP	Investigational Product
IVRS	Interactive Voice Response System
IWRS	Interactive Web Response System
LSLV	Last Subject Last Visit
OAE	Other Significant Adverse Event
PGx	Pharmacogenetic research
PI	Principal Investigator
SAE	Serious adverse event
WBDC	Web Based Data Capture

1. INTRODUCTION

1.1 Background and rationale for conducting this study

Platelet activation and aggregation in response to atherosclerotic plaque rupture is a key event in the pathogenesis of acute myocardial infarction (AMI) [1]. Platelet P2Y12 receptors for adenosine diphosphate are essential for platelet activation [2], and from this reason antagonists against the P2Y12 receptor are established in secondary prevention of cardiovascular events [3]. Beyond blocking platelet activation, antagonists of the P2Y12 receptor have hitherto unexplained anti-inflammatory effects [4,5]. Findings from the PLATelet inhibition and patient Outcomes (PLATO) study demonstrated that platelet inhibition with a novel and potent P2Y12 receptor antagonist ticagrelor reduced mortality following pulmonary infections and sepsis compared to clopidogrel, previous standard treatment for patients with AMI [5].

Activated platelets release platelet extracellular vesicles (PEV) [6]. PEV differ from other biomarkers of inflammation and platelet activation, because (i) PEV are released from activated platelets at the early stage of developing arterial thrombosis [7], (ii) PEV expose specific platelet antigens and thus can be identified as being platelet-specific [8], (iii) PEV participate both in inflammation and in coagulation due to exposure of P-selectin and phosphatidylserine (PS) [9,10]. P-selectin initiates binding of platelets and/ or PEV to leukocytes which leads to leukocyte activation and secretion of cytokines [9], and (ii) PS and other negatively charged phospholipids bind positively charged clotting factors thereby propagating thrombin generation [10]. Additionally, PEV serve as pro-inflammatory stimuli in acute lung injury [11], which is one of the leading causes of death in sepsis [12]. Because identification and characterization of PEV directly in human plasma has recently become feasible [13], and because PEV can be measured in biorepositories thereby facilitating validation in multicentre clinical trials [14], it is likely that the requirements for PEV to become a reliable biomarker can now be fulfilled.

1.2 Rationale for study design, doses and control groups

Rationale for study design

Clinical studies suggest that platelet P2Y12 receptor antagonists exert anti-inflammatory effects [4,5], but the underlying mechanisms have not been clearly defined *in vivo*. This study is expected to identify an additional mechanism of action of the P2Y12 antagonist ticagrelor (inhibition of PEV release), which might partly explain the lower rate of infection-related mortality in patients treated with ticagrelor, compared to clopidogrel [5]. Regarding the exponentially growing interest in the field of EV in the last decade [6,13,14], as well as the crucial role of PEV in inflammation and thrombosis [9,10], determining the effect of ticagrelor on PEV release is an important step in cardiovascular pharmacology research. Although the study is preliminary in the design and not powered for mortality, the results are expected to pave the road for further studies designed to investigate the association between concentrations of PEV and infection-releated mortality in patients treated with ticagrelor, and facilitate the development of PEV-based pharmaceuticals with applications in atherosclerosis-related cardiovascular diseases.

The randomised and investigator-blinded allocation to the study groups along with state-of-the art methods of PEV analysis will account for the reliability of results. The study will be conducted in the 1st Chair and Department of Cardiology, Medical University of Warsaw, Poland. The analytical part will be conducted in the Vesicle Observation Centre, Academic Medical Centre, University of Amsterdam, The Netherlands. The involvement of experts with a track record on extracellular vesicles from the Netherlands (Dr. Rienk Nieuwland, Prof. Auguste Sturk) will ensure a scientifically sound project and optimize dissemination of results.

Doses

All drugs will be administered orally, in doses recommended by European Society of Cardiology in the Guidelines on Myocardial Revascularization [3]. If not already administered prior to hospital admission, patients will receive a loading dose of clopidogrel (600 mg) prior to percutaneous coronary intervention (PCI). Patients randomised to ticagrelor will receive a loading dose of ticagrelor (180 mg), followed by a maintenance dose (90 mg twice daily).

Patients randomised to clopidogrel will continue the treatment with a maintenance dose of clopidogrel (75 mg once daily).

Control group

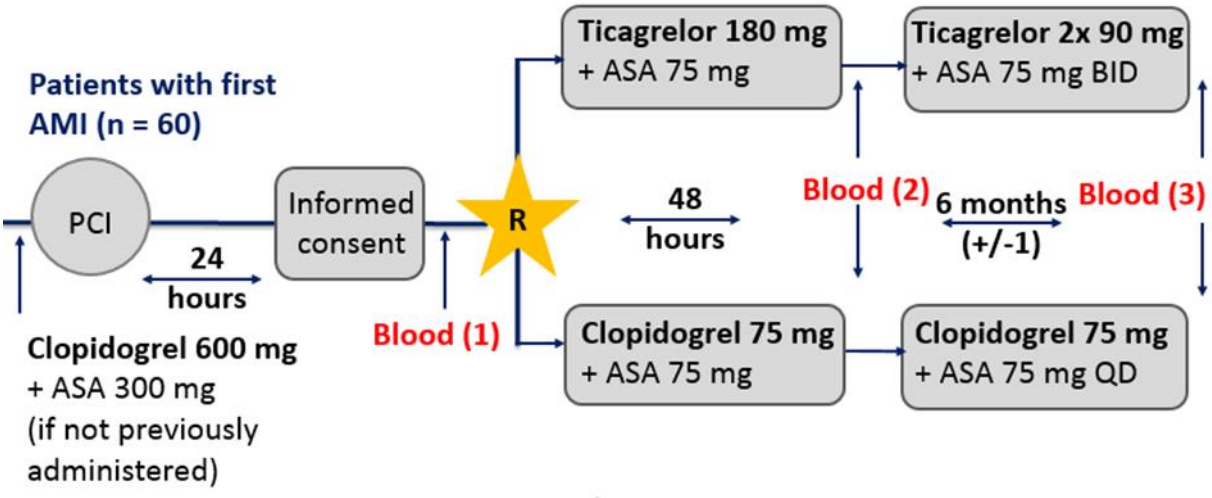
- Patients randomised to clopidogrel.

Benefit/risk and ethical assessment

All study drugs are administered on-label. According to the Guidelines on Myocardial Revascularization, both in subjects with STEMI and NSTEMI ticagrelor is recommended over clopidogrel, and clopidogrel is recommended when ticagrelor is not available or is contraindicated [3]. Because ticagrelor is not reimbursed in Poland, it is not available for the majority of Polish patients due to financial constraints. Participation in the study ensures the free access to ticagrelor for patients randomized to the study group during the first 6 months after AMI, which is the period of the highest risk of recurrent major adverse cardiovascular events (MACE) [15,16]. Participation in the study ensures also the free access to clopidogrel for patients randomized to the control group during the first 6 months after AMI. All patients will undergo an additional cardiovascular investigation at the last visit at 6 months.

1.3 Study Design

Figure 1. Study flow chart.



Blood: Platelet extracellular vesicles, aggregometry

Abbreviations: AMI – acute myocardial infarction; PCI – percutaneous coronary intervention; ASA – acetylsalicylic acid; R – randomization, QD – once daily; BID – twice daily

2. STUDY OBJECTIVES

2.1 Primary objective

Primary Objective:	**Outcome Measure:**
To compare the effects of ticagrelor and clopidogrel on the concentration of PEVs in patients with acute myocardial infarction.	Concentration of PEV exposing P-selectin and PS (flow cytometry).

2.2 Secondary objectives

Secondary Objective:	**Outcome Measure :**
To compare the effects of ticagrelor and clopidogrel on the concentration of EVs exposing fibrinogen, exposing phosphatidylserine, EVs from endothelial cells, EVs from leukocytes.	Concentration of EV exposing fibrinogen, exposing phosphatidylserine, EVs from endothelial cells, EVs from leukocytes.

2.3 Safety objectives

Safety Objective:	**Outcome Measure :**
To evaluate the safety and tolerability of ticagrelor in relation to clopidogrel. To be reported descriptively.	- Vital signs - Abnormal values in blood tests (haematology and clinical chemistry) - Abnormal values or in urinalysis - Abnormalities in 12-lead ECG - AEs/ SAEs

2.4 Exploratory objectives

Exploratory Objective:	Outcome Measure :
To identify an additional mechanism of action of ticagrelor, which may at least in part explain the lower rate of infection-related mortality in patients treated with ticagrelor, compared to clopidogrel.	Concentration of PEV exposing P-selectin and phosphatidylserine (PS).

3. SUBJECT SELECTION, ENROLMENT, RANDOMISATION, RESTRICTIONS, DISCONTINUATION AND WITHDRAWAL

3.1 Inclusion criteria

- Age > 18 years
- Informed consent to participate in the study
- PCI due to first ST-elevation myocardial infarction (STEMI) or non-STEMI (NSTEMI)
- Administration of the loading dose of clopidogrel (600 mg) prior to PCI

3.2 Exclusion criteria

- Known coagulopathy
- Active pathological bleeding
- Known history of bleeding disorder
- Suspicion of intracranial haemorrhage
- Need for oral anticoagulation therapy
- Administration of GPIIb-IIIa antagonists
- Cardiogenic schock
- Severe chronic renal failure (eGFR < 30 mL/min)
- Severe liver insufficiency
- Infectious disease
- Autoimmune disease

- Neoplasm
- Chronic dyspnea
- Increased risk of bradycardia
- Known pregnancy, breast-feeding, or intention to become pregnant during the study period
- Study drug intolerance
- Co-administration of ticagrelor or clopidogrel with strong CYP3A4 inhibitors
- Participation in any previous study with ticagrelor or clopidogrel

3.3 Subject enrolment and randomization

Subjects will be enrolled by the PI at the 1st Chair and Department of Cardiology, Medical University of Warsaw. Subjects will be randomized by an independent operator at 1st Department of Cardiology, Medical University of Warsaw. Randomisation will be carried out in 1:1 ratio using block randomization via the mobile application “Randomizer for Clinical Trial” (Medsharing, France).

3.4 Procedures for handling incorrectly enrolled or randomized subjects

Subjects incorrectly enrolled or randomized subjects will be withdrawn from the study. These subjects:

- will be apologized,
- will be administered double antiplatelet therapy with ASA and a P2Y12 antagonist, as well as other medications, if required,
- will be asked to return the study medication, if identified after the study medication has already been dispensed,
- will be monitored in the same way as subjects continuing the study until the last visit at 6 months.

The PI will maintain a list of incorrectly enrolled or randomized subjects in the Investigator's Study File.

3.5 Methods for assigning treatment groups

Upon successful randomization, the subject will be assigned a randomization number and treatment. The subject’s identification data and ID number, and randomization number will be

listed on the Patient Identification List and Patient Enrolment List, respectively, and stored in the Investigator's Study File.

3.6 Methods for ensuring blinding

Not applicable to study subjects. The PI will be blinded to the randomization arm and treatment of study subjects. The operator analyzing the samples will be blinded to all subject-related baseline and clinical data.

3.7 Methods for unblinding

Not applicable for patients. The PI will be unblinded by combining the Patient Identification List including subjects identification data and Patient Enrolment List including data on subjects randomization

3.8 Restrictions.

Not stated.

3.9 Discontinuation of investigational product

The investigational product (IP) may be discontinued at any time at the discretion of the PI or the study subject. Criteria for discontinuing the investigational product include:

- Withdrawal of informed consent.
- Development of exclusion criteria, pregnancy or other safety reasons during the study (requirement of unacceptable concomitant medication, adverse event, intolerance of the IP)
- Protocol non-compliance.
- Incorrect enrolment or randomization of the subject.

3.9.1 Procedures for discontinuation of a subject from investigational product

For subjects discontinued from the IP, the same measurements and assessments will be performed as in patients continuing the study until the last visit at 6 months, including monitoring of the adverse events. The subjects will be asked to return the study medication.

3.10 Criteria for withdrawal

Criteria for withdrawal from the study include:

- Withdrawal of informed consent.

- Development of exclusion criteria, pregnancy or other safety reasons during the study (requirement of unacceptable concomitant medication, adverse event, intolerance of the investigationa l product).
- Protocol non-compliance.
- Incorrect enrolment or randomization of the subject.
- Lost to the last visit at 6 months.
- Any other reason, in the investigator's opinion, that would impede the subject's participation in the study

  The withdrawn subjects will be replaced by subjects subsequently recruited for the study, until the estimated sample size of the study participants will be achieved.

3.10.1 Screen failures

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

3.10.2 Withdrawal of the informed consent

Subjects are free to withdraw from the study at any time for any reason. Should a subject withdraw a consent, every effort will be made to complete and report the observations as thoroughly as possible.

3.11 Discontinuation of the study

The Sponsor or PI may discontinute the study prematurely at any time due to the following reasons:

- the safety of the participants is doubtful or at risk,
- alterations in accepted clinical practice that make the continuation of a clinical trial unwise,
- early evidence of benefit or harm of the experimental intervention
- insufficient participant recruitment.

4. STUDY PLAN AND TIMING OF PROCEDURES

Visit	**1**	**2**	**3**	**4**	**5**		**No follow-up**	For details see Protocol Section
Visit window Day –1 to 0 for Visit 1 ±1 day for Visit 2 ±3 days for Visit 3 ±5 days for Visit 4 ± 6 months for Visit 5								
Week		**0**	**1**			**24**		
Day	**-**	**0**	**1**	**3**	**5**	**168**		
Written informed consent	X							
Demographics	X							
Physical examination, height, and weight	X		X	X	X	X		

Visit	**1**	**2**	**3**	**4**	**5**		**No follow-up**	For details see Protocol Section
Visit window Day –1 to 0 for Visit 1 ±1 day for Visit 2 ±3 days for Visit 3 ±5 days for Visit 4 ± 6 months for Visit 5								
Week		**0**	**1**			**24**		
Day	**-**	**0**	**1**	**3**	**5**	**168**		
Medical/surgical history	X							
Inclusion/exclusion criteria	X	X						
12-lead ECG	X		X	X	X	X		
Vital signs	X	X	X	X	X	X		
Randomisation to study treatment		X						
Treatment dispensed/returned		X				X		
Concomitant medication	X	X	X	X	X	X		
Adverse event review (AEs and SAEs)		X	X	X	X	X		

Visit	**1**	**2**	**3**	**4**	**5**		**No follow-up**	For details see Protocol Section
Visit window Day –1 to 0 for Visit 1 ±1 day for Visit 2 ±3 days for Visit 3 ±5 days for Visit 4 ± 6 months for Visit 5								
Week		**0**	**1**			**24**		
Day	**-**	**0**	**1**	**3**	**5**	**168**		
Blood samples for haematology and clinical chemistry	X			X		X		
Urinalysis	X			X		X		
Blood sampling for pharmacokinetics		n.a.	n.a.	n.a.	n.a.	n.a.		

4.1 Enrolment/screening period

6 months

4.2 Treatment period

6 months

4.3 Follow-up period

No follow-up period after the end of the study treatment.

5. STUDY ASSESSMENTS

5.1 Efficacy assessments

The efficacy of ticagrelor will be assessed as a decrease in the concentration of EVs, in comparison with clopidogrel.

5.2 Safety assessments

5.2.1 Laboratory safety assessments

Table x Laboratory Safety Variables

Haematology/Haemostasis (whole blood)	Clinical Chemistry (serum or plasma)
B-Haemoglobin (Hb)	S/P-Creatinine
B-Leukocyte count	S/P-Aspartate transaminase (AST)
B-Platelet count	S/P-Alanine transaminase (ALT)
	S/P-Potassium
	S/P-Sodium
Urinalysis (dipstick)	
U-Hb/Erythrocytes/Blood	
U-Protein/Albumin	
U-Glucose	

5.2.2 Physical examination

A comprehensive physical examination consisting of observation, percussion, palpation and auscultation will be conducted with state-of-the art questionnaire commonly used at 1st Chair and Department of Cardiology. The following will be examined: general appearance, skin (colour, hydration, oedema, scars, spots, haemorrhagic manifestations) , head and neck (eyes, ears, nose, mucous membranes, lymph nodes, thyroid gland), cardiovascular system (heart, carotid arteries, peripheral arteries), respiratory tract (chest, lungs), abdomen (peristaltic sounds, palpation of the abdominal organs including liver and spleen), neurological system (cerebral functions, cerebellum tests, motor and sensory nerves, cranial nerves, reflexes), musculoskeletal system (skeletal abnormalities, mobility).

5.2.3 ECG

The ECG report will include heart rhythm, heart rate, heart axis, P wave, PR interval, QRS complex, ST segment, T wave, and QT interval.

5.2.3.1 Resting 12-lead ECG

Resting 12-lead ECG will be performed using state-of-the-art ECG instrument available at 1st Chair and Department of Cardiology.

5.2.4 Vital signs

The following vital signs will be examined: body temperature, respiratory rate, pulse rate, blood pressure.

5.2.4.1 Pulse and blood pressure

Pulse and blood pressure will be measured at both arms during the first visit. If there is a difference less than 10 mmHg in systolic blood pressure between the arms, blood pressure will be measured at the non-dominant arm. Otherwise blood pressure will be measured at the arm with the higher blood pressure. The measurement will be done digitally using the instruments available at 1st Chair and Department of Cardiology.

5.2.4.2 Body temperature

Body temperature will be measured at the forehead using digital thermometer available at 1st Chair and Department of Cardiology.

5.2.5 Other safety assessments

Chest x-ray and echocardiography will be conducted as other safety assessments.

5.3 Other assessments

5.3.1 Patient reported outcomes (PRO)

PROs will be collected via paper forms and kept in the Investigator's Study File. The following measures will be collected as PRO:

- Symptoms (cardiovascular, respiratory, gastrointestinal, urogenital, neurological, musculoskeletal),
- Health-related quality of life (HRQL)

5.3.1.1 Name of PRO method or questionnaire

- State-of-the art used at 1st Chair and Department of Cardiology to assess symptoms.
- SF-12 Health Survey validated in patients after myocardial infarction to assess quality of life [17].

5.4 Pharmacokinetics

Not applicable

5.5 Pharmacodynamics

Not applicable.

5.6 Pharmacogenetics

Not applicable

5.7 Biomarker analysis

Concentrations of PEV and other biomarkers will be analysed in citrate-anticoagulated plasma prepared according to recently standardized protocols for handling and storage of human body fluids for analysis of extracellular vesicles [14,18]. Venous blood will be collected from fasting individuals to measure concentrations of EVs and platelet aggregation. The protocol for blood collection and handling is enclosed in Appendix D. Blood sampling will be conducted in all subjects three times: (i) 24 hours following administration of loading dose of clopidogrel, (ii) 48 hours following randomisation to ticagrelor or clopidogrel group, and (iii) 6 months following the index hospitalization. PEV analysis

- Concentrations of PEVs will be determined by state-of-the art flow cytometry (Apogee A60 Micro, UK) [14]. A selection of typical PEV samples will be isolated by size-exclusion chromatography [19] and analysed by transmission electron microscopy to confirm the presence of PEV and to study PEV morphology.
- Platelet function

Platelet function will be assessed by Multiplate impedance aggregometry (Multiplate Analyzer, Roche Diagnostics, Germany) using adenosine diphosphate (ADP; 6.5 μM) and thrombin receptor-activating peptide (TRAP, 32 μM) as agonists. Methodology applied to measure platelet function enables rapid and reliable on-site evaluation of platelet function,

including monitoring of platelet inhibition in patients treated with the P2Y12 receptor antagonists [20].

5.7.1 Storage, re-use and destruction of biological samples

All samples will be stored at 1st Chair and Department of Cardiology in a freezer, at the temperature of -80°C, under continuous temperature monitoring. In case of the freezer breakdown, the samples will be immediately transported to the nearby Centre for Preclinical Research and Technology (5 minutes walking distance). Only one freeze-thaw cycle will be permitted to analyse biomarkers. The re-use of thawed samples will not be permitted in course of the study. Every effort will be made to avoid destruction of stored samples.

5.7.2 Labelling and shipment of biological samples

All collected samples will be labelled with a unique code number prepared using Brady Label Printer (Brady, US) and analysed in one block by an operator blinded to patient- and treatment-related data. All samples will be transported on dry ice to Vesicle Observation Centre, Academic Medical Centre, University of Amsterdam, The Netherlands for analysis.

5.7.3 Chain of custody of biological samples

The PI or the study nurse will withdraw the blood with strict adherence to the protocol. The PI will prepare the plasma and transport the samples for storage (2 minutes walking distance). The PI will be the only person handling the samples to minimize the risk of sample handling variability, thereby maximizing the reliability of obtained results. The samples will be stored as described in the point 5.7.1. of the protocol until shipped to the Vesicle Observation Centre, Academic Medical Centre, University of Amsterdam for analysis.

5.7.4 Withdrawal of Informed Consent for donated biological samples

Subjects are free to withdraw the Informed Consent for donated biological samples at any time for any reason. The donated biological samples from subjects who withdrew a consent will be excluded from analysis.

6. SAFETY REPORTING AND MEDICAL MANAGEMENT

6.1 Definition of adverse events

Adverse events (AE) and serious adverse events (SAE) will be defined according to the guidelines of Food and Drug Association. AE will be defined as the development of an undesirable medical condition or the deterioration of a pre-existing medical condition occurring during the study in a subject administered a pharmaceutical product which does not necessarily has a causal relationship with the product. SAE will be defined as any AE which fulfil one or more of the following criteria:

- Results in death;
- Is immediately life-threatening;
- Requires hospitalization;
- Results in persistent or significant disability or incapacity.
- Is a congenital anomaly/ birth defect.

The following hospitalisations will not be considered to be SAE:

- Those planned before entry into the study;
- Elective treatment for a condition unrelated to study indication or study treatment;
- Occurred on an emergency outpatient basis and do not result in admission (unless fulfilling other criteria in SAE definition);
- Part of normal treatment or monitoring of the study indication and are not associated with any deterioration in condition.

6.2 Recording of adverse events

AE will be collected by means of a standard question: “Have you had any health problems since the previous visit?” Reported AE’s and/ or observed AE’s will be recorded on the AE Form. The AE Forms will be stored in Investigator's Study File.

6.1.1 Time period for collection of adverse events

AE and SAE will be analyzed at each visit during index hospitalization and at the last visit at 6-months.

6.1.2 Follow-up of unresolved adverse events

Every effort will be made to follow-up and resolve the unresolved AE.

6.1.3 Variables

The following variables will be collected for each AE;

- Description of AE.
- The date and time when the AE started and stopped.
- The intensity of the AE according as assessed by study subjects according to the following scale:
  - Mild = awareness of sign or symptom, but easily tolerated
  - Moderate = discomfort sufficient to cause interference with normal activities
  - Severe = incapacitating, with inability to perform normal activities.
- The intensity of the AE assessed by PI as CTCAE grade 1-5.
- Whether the AE is serious or not. The AE assessed by the study subject as severe AE is not equivalent to serious AE.
- Causality assessment in relation to study treatment.
- Action taken with regard to investigational product.
- Withdrawal of the subject from the study due to AE.
- Outcome.

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

- Date when AE met criteria for serious AE.
- Date Investigator became aware of serious AE.
- The reason why AE is serious.
- Date of hospitalization.
- Date of discharge or death.
- Probable cause of death.
- Autopsy performed.

6.1.4 Causality assessment

The causality of AE in relation to study treatment will be assessed by the PI, who in completing the relevant AE Report Form will 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 study medication?" Causality will be assessed using the following definitions:

- Very likely
  - The AE follows a reasonable temporal sequence from study treatment administration;
  - Abates upon discontinuation of study treatment;
  - Reappears on repeated exposure (re-challenge).
- Probable
  - The AE follows a reasonable temporal sequence from study treatment administration;
  - Abates upon discontinuation of study treatment;
  - Cannot reasonably be explained by known characteristics of the subject's clinical state.
- Possible
  - The AE follows a reasonable temporal sequence from study treatment administration, but could have been produced by the subject's clinical state or other mode of therapy administered to the subject.
- Doubtful
  - The temporal association between study treatment and AE is such that the study treatment is not likely to have any reasonable association with the observed event.
- Very unlikely
  - The AE is definitely produced by the subject's clinical state or other mode of therapy administered to the subject.

6.1.5 Disease progression

Not applicable for the purpose of the study.

6.2 Reporting of serious adverse events

Information about all SAE will be recorded on the SAE Form in Investigator's Study File. All events documented in the SAE Form will be reported within 24 hours to the Sponsor (personally or via fax) and to the Office of Medicinal Products, Medicinal Devices and Biological Products (via the online form available at www.urpl.gov.pl). All SAEs are to be submitted to the AstraZeneca Product Safety mailbox: AEMailboxClinicalTrialTCS@astrazeneca.com

6.3 Overdose

A subject with confirmed or suspected overdose of the study medications will undergo thorough clinical and laboratory investigation. In case of any abnormalities upon the investigation, the subjects will be admitted to 1st Chair and Department of Cardiology for further assessment and monitoring.

6.4 Pregnancy

6.4.1 Maternal exposure

The female subject will be instructed to inform the PI if she becomes pregnant during the study and seek advice regarding continuation of the study treatment. The PI will report the pregnancy to the Sponsor within 24 hours of being notified of the pregnancy. The pregnancy will be followed up until the outcome is known.

6.4.2 Paternal exposure

Pregnancy occurring in the partner of a male subject participating in the study will also be will be counselled as described above, reported to the Sponsor and followed up until the outcome is known.

6.5 Management of toxicities <<Dose Reductions>>

A subject with confirmed or suspected toxicity of the study medications will be admitted to 1st Chair and Department of Cardiology for assessment and monitoring. After considering of

benefit to risk ratio the drug will be temporarily withheld. If the toxicity continues after re-initation of the treatment, the drug will be permanently withheld.

6.6 Study governance and oversight

6.6.1 Steering Committee

The Steering Committee will regularly discuss the course and the quality of the study. The Steering Committee includes Prof. Filipiak (Vice-Rector of Medical University of Warsaw and a Study Coordinator), Prof. Opolski (Head of the 1st Chair and Department of Cardiology of Medical University of Warsaw), Prof. Auguste Sturk (Head of the Department of Clinical Chemistry of Academic Medical Centre of University of Amsterdam), Dr. Rienk Nieuwland (Head of the Vesicle Observation Centre of Academic Medical Centre of University of Amsterdam), and PI (cardiology resident and PhD student at the 1st Chair and Department of Cardiology of Medical University of Warsaw).

6.6.2 Data Monitoring Committee

There is a Monitoring Committee at the study site which monitors the Investigator's Study File to verify adherence to the protocol and the completeness, consistency and accuracy of the data. The investigator agrees to cooperate with the Monitoring Committee to ensure that any problems detected in the course of these monitoring visits are resolved.

6.6.3 Scientific Advisory Committee

The Scientific Advisory Committee consists of the same members as the Steering Committee excluding PI. The PI will regularly discuss the questionable issues related to the study with the Scientific Advisory Committee.

7. INVESTIGATIONAL PRODUCT AND OTHER TREATMENTS

7.1 Identity of investigational product(s)

Investigational product	Dosage form and strength	Manufacturer
Ticagrelor	Tablets, 90 mg	AstraZeneca
Clopidogrel	Tablets, 75 mg	Synoptis Pharma

7.2 Dose and treatment regimens

The investigational product ticagrelor will be administered orally in a loading dose 180 mg, followed by a maintenance dose of 90 mg twice daily for 6 months. Clopidogrel will be administered orally in a loading dose 600 mg, followed by a maintenance dose of 75 mg twice daily.

7.3 Labelling

Each study drug box will be labelled with the following information:

- Sponsor identification
- Manufacturer's identification
- Protocol number
- Caution statement
- 'For Clinical Trial Use Only' statement
- Investigational product identification and batch number
- Quantity of bottles
- Storage conditions
- Expiry date

7.4 Storage

The investigational product will be stored in accordance with the manufacturers' instructions. Required conditions for storage will be printed on the medication label. The temperature within the load will be measured at least twice daily and recorded in the Investigator’s Study File. Investigational product will be kept under adequate security by the PI and only accessible to authorised study personnel.

7.5 Compliance

Each subject will be dispensed sufficient medication for 24 weeks of therapy. Compliance will be checked by counting of blisters at the last visit at 6 months.

7.6 Accountability

The PI will maintain current record of the receipt, inventory and dispensing, including shipping invoices, of all study supplies. The Investigational Product Accountability File will include:

- Date received
- Reference number and amount received and placed in storage
- Name of study medication and dosage
- Label ID number or batch number
- Name of person responsible for each investigational product inventory entry
- Amount dispensed to and returned by each subject, including unique subject identifiers
- Non-study disposition (e.g. lost, wasted, broken)
- Amount returned to Sponsor

Accountability files will be available for inspection by the monitor at any time. Upon completion/ termination of the study, unused investigational product will be returned to the Sponsor reconciliation.

7.7 Concomitant and other treatments

Resticted Medication/Class of drug:	
Non-steroid anti-inflammatory drugs Oral anticoagulants	

Prohibited Medication/Class of drug:	

Ketoconazole/ antifungal drugs Clarithromycin/ antibiotics Ritonavir/ antiretroviral medications Other strong CYP3A4 inhibitors	

7.7.1 Other concomitant treatment

Subjects will receive a loading dose of aspirin (300 mg) prior to PCI. Unfractionated heparin will be administered during PCI in the dosage left at the discretion of interventional cardiologist. On discharge, all subjects will receive treatment with ASA 75 mg once daily, atorvastatin at least 10 mg once daily, and angiotensin-converting enzyme (ACE)-inhibitor or angiotensin II receptor antagonist (ARB). If indicated, subjects will receive treatment with β-receptor antagonist and mineralocorticoid-receptor antagonist.

7.8 Post Study Access to Study Treatment

According to the guidelines, double antiplatelet therapy with ASA and a P2Y12 receptor antagonist will be continued for 12 months. Because the study duration is 6 months, the study treatment will be continued until last visit at 6 months. After termination of the study patients will be prescribed double antiplatelet therapy for the remaining 6 months. Ticagrelor will be recommended over clopidogrel. Patients who choose to switch to clopidogrel due to financial constraints will be prescribed clopidogrel.

8. STATISTICAL ANALYSES

8.1 Statistical considerations

A single statistical analysis will be performed at the end of the study by an independent professional statistician from the Epidemiology Department of Medical University o Warsaw using IBM SPSS Statistics 20 and GraphPad Prism 5. An intention to treat (ITT) approach

will followed, i.e. statistical analysis will be based on data from all patients who were randomized and from whom meaningful data were collected. Data will be displayed graphically for visual inspection. Categorical variables will be presented as number and percent; continuous variables will be presented as mean and standard deviation (SD) or median with inter-quartile range. Shapiro–Wilk test will be used to test for non-Gaussian distribution of continuous variables.

8.2 Sample size

Because there is no sufficient data to assess the impact of ticagrelor on the concentrations of PEV in patients with AMI, the SD and mean difference between the groups was estimated based on the preliminary experiments, which we conducted in during preparation for this study. The required sample size was calculated by a two-sided t-test at a significance level of 0.05 with the following assumptions: (i) SD in each group ± 1, (ii) mean difference between the groups = 1, (iii) nominal test power = 0.9. Based on this sample size estimation, it is calculated that a total of 60 patients will be enrolled in the trial. The conference abstract [15], as well as the explanation on how the sample size has been calculated are included in Appendix E.

8.3 Definitions of analysis sets

The following sets of analysis will be performed: analysis of baseline characteristics, efficacy analysis, PRO analysis.

8.3.1 Efficacy analysis set

The efficacy of ticagrelor will be assessed as decrease in concentration of PEVs (flow cytometry), in comparison with clopidogrel.

8.3.2 Safety analysis set

AE reported throughout the course of the study including pre-study, study and post-study findings of physical examination, vital signs, laboratory variables, and 12-lead ECG will be listed individually per treatment group and analyzed. Due to the fact that the study is not

powered for clinical endpoints, AE will be analyzed as safety events and reported descriptively.

8.3.3 PK analysis set

Not applicable.

8.3.4 PRO analysis set

PRO reported throughout the course of the study including findings of the standard symptoms questionnaire used at 1st Chair and Department of Cardiology and SF-12 questionnaire will be listed individually per treatment group and analyzed.

8.3.5 Analysis of the primary variable(s)

Primary variable is the concentration of PEV exposing P-selectin and PS. Student's two-sided t-test or Mann–Whitney U test will be used to compare the concentrations between the two treatment arms. ANOVA-repeated measures or Friedman test will be used to compare the concentrations between the three blood samplings for PEV analysis within each group. A p-value below 0.05 will be considered significant.

8.3.6 Analysis of the secondary variable(s)

Secondary variables are the concentration of EVs exposing fibrinogen, exposing phosphatidylserine, EVs from endothelial cells, EVs from leukocytes (flow cytometry(and platelet aggregation (Multiplate impedance aggregometry). Student's two-sided t-test or Mann–Whitney U test will be used to compare the concentrations between the two treatment arms. ANOVA-repeated measures or Friedman test will be used to compare the concentrations between the three blood samplings for PEV analysis within each group. A p-value below 0.05 will be considered significant.

8.3.7 Subgroup analysis (if applicable)

Not applicable.

8.3.8 Interim analysis

Not applicable.

8.3.9 Sensitivity analysis (if applicable)

Not applicable.

8.3.10 Exploratory analysis (if applicable))

Not applicable.

9. STUDY AND DATA MANAGEMENT

9.1 Training of study site personnel

The PI attented a 4-week-long internship at the Vesicle Observation Centre of Academic Medical Centre of University of Amsterdam, where she was trained in protocols of collection and handling of body fluids for analysis of extracellular vesicles, as well as methods to isolate and detect EV with EV-dedicated flow cytometry (Apogee A60-Micro), enzyme-linked immunosorbent assay (ELISA), single particle detection methods such as Nanoparticle Tracking Analysis (NTA) and Tunable Resistive Pulse Sensing (TRPS). The PI will transfer her knowledge and skills to the study site personnel, which includes two other investigators and a study nurse. All samples throughout the study will be handled by a PI exclusively to minimize the investigator-related variability and maximize the reliability of the results.

9.2 Monitoring of the study

9.2.1 Source data

Source data include original hospital records, clinical charts, original laboratory reports, memoranda, pharmacy dispensing records, recorded data from automated instruments, x-rays, magnetic or electronic media, and all other documents stored in subjects' individual files.The

PI will maintain source documents for each patient in the study. The information in the Investigator's Study File will be traceable to these source documents.

9.2.2 Study agreements

The Sponsor declare to sign the confidential agreement and the study agreement with AstraZeneca Pharma Poland and to conduct the study according to signed agreements.

9.2.3 Archiving of study documents

The source data will be archived according to the local legal and regulatory requirements of 1st Chair and Department of Cardiology, Medical University of Warsaw.

9.3 Study timetable and end of study

Project IEC approval	May 10, 2016
First Subject In	December 28, 2016
50% Enrollment	March 28, 2017
Last Subject In (100% enrollment)	June 28, 2017
Last Subject Last Visit (Treatment end)	December 28, 2018
Publication	June 28, 2019

Data management will be conducted using database created for the study and stored on the validated platform for data protection of Academic Medical Centre, University of Amsterdam. Accurate and reliable data collection will be assured by verification and cross-check of the Investigator's Study File against the source documents. Investigator's Study File will be maintained including at least the following documents/information:

- Signed protocol and amendments
- Sample case report forms (CRF)
- Current informed consent form and all revisions
- Current patient information sheet and all revisions
- Financial aspects of the study

- All signed agreements/contracts
- Dated, documented approval of ethics committee and regulatory authorities
- Monitoring reports
- Reports to ethics committee and regulatory authorities
- Notification by sponsor of safety information
- Screening Failure List
- Incorrectly enrolled or randomized subjects list
- Patient Enrolment List.
- Patient Identification List
- Patient reported outcomes
- AE Forms and SAE Forms
- Investigational Product Accountability File

Adverse Event (AE) Reconciliation

After termination of the study the AE Reconciliation will be performed.

Data Management of genotype data

No genotype data will be collected throughout the study.

Data associated with human biological samples

Only the PI, study site personnel and statistician will have access to data associated with human biological samples. The server hosting the platform is recurred with personal passwords.

Management of external data

Only the PI, study site personnel, statistician, and treating physician will have access to external data. The external data will be protected and archived according to the local data policy.

10. ETHICAL AND REGULATORY REQUIREMENTS

10.1 Ethical conduct of the study

The study will be conducted in compliance with the protocol, GCP, the ethical principles described in the Declaration of Helsinki, the requirements of the European Medicines Agency, and local legal and regulatory requirements.

10.2 Subject data protection

The PI will assure that the subjects' anonymity will be maintained and that the confidentiality of records and documents that could identify subjects will be protected, respecting the privacy and confidentiality rules in accordance with applicable regulatory requirements. Before unblinding of the PI, subjects will be identified only by their assigned identification number and initials. A co-investigator will keep a Patient Identification List with complete identification information (name, address, contact number).

10.3 Ethics and regulatory review

The study protocol was approved by the IEC of Medical University of Warsaw in May 2016 (approval number: KB/112/2016, amendment: KB/79/A/2016, Appendix B), and is registered on ClinicalTrials. The PI will inform IEC of Medical University of Warsaw about any serious and/or unexpected events occurring during the study, and any new information that may adversely affect the safety of the subjects or the conduct of the study. All correspondence with the IEC of Medical University of Warsaw will be stored by the PI in the Study.

10.4 Informed consent

The PI will inform every subject in detail about the nature of the study, its purpose, the treatments and the probability of random assignment to treatment groups. Every subject will receive information sheet and provide written informed consent prior to participation the study. A copy of the Patient Information Sheet and signed Consent Form will be given to the subject. The original will be stored by the PI in the Investigator's Study File. A sample of the

Patient Information Sheet and Consent Form can be found in the Appendix C of this protocol (in Polish). The study subjects will also be informed that:

- Participation in this study is voluntary and the informed consent to participate in the study may be withdrawn at any time for any reason, and that withdrawal of consent will not affect the subsequent medical treatment or relationship with the treating physician.
- They will be notified if information becomes available that may be relevant to their willingness to continue participation in the study.
- They will be notified if alternative treatment becomes available, as well as about the potential benefits and risks of this alternative treatment.
- They will be strongly encouraged to contact the PI to report on any potentially study-related adverse events, as well as for further information regarding the study.

10.5 Changes to the protocol and informed consent form

The PI will inform IEC of Medical University of Warsaw about any amendment to the protocol, informed consent changes or revisions of other documents originally submitted for review.

10.6 Audits and inspections

Auditors and inspectors from Sponsor and representatives of IEC or other regulatory agencies will be granted direct access to subject medical records and other study documents for verification of study procedures and data without violating the confidentiality of the subject. The subject should be informed that by signing a written informed consent form that he or she is authorizing such access.

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