Comparison of Pharmacodynamic Effects of Tirofiban vs. Cangrelor in NSTEMI Patients Undergoing Percutaneous Coronary Intervention

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Synopsis

Title: Comparison of Pharmacodynamic Effects of Tirofiban vs. Cangrelor in NSTEMI Patients Undergoing Percutaneous Coronary Intervention

Short Title: Tirofiban vs. Cangrelor in NSTEMI

Rationale: Immediate potent inhibition of platelet function is critical for the prevention of periprocedural ischemic event occurrences in high risk N-ST segment elevation myocardial infraction (NSTEMI) patients undergoing percutaneous coronary intervention (PCI). Currently, dual antiplatelet therapy with aspirin and an oral $P2Y_{12}$ receptor blocker (with loading doses) is widely used for PCI.[1] However, immediate, potent and reversible inhibition of platelet aggregation is not possible even with the newer oral agents, prasugrel and ticagrelor. Therefore, an intravenously administered GPIIb/IIIa receptor inhibitor (tirofiban) or $P2Y_{12}$ receptor blocker (cangrelor) with fast onset and offset of actions will provide more desired antiplatelet effects in the setting of PCI. Cangrelor provides effective inhibition of only ADP-mediated upstream platelet activation and aggregation pathways via selective inhibition of the $P2Y_{12}$ receptor whereas a GPIIb/IIIa inhibitor such as tirofiban, will provide effective platelet inhibition by inhibiting the final common pathway of platelet aggregation stimulated by multiple agonists such as collagen, thrombin receptor activator peptide (TRAP), arachidonic acid (AA) and adenosine diphosphate (ADP). The latter may translate in to superior clinical benefits compared to cangrelor therapy.[2,3]

Objectives:
Primary Objective: Assessment of platelet aggregation in response to thrombin receptor activator peptide (TRAP) at baseline and serially following tirofiban or cangrelor infusion.

Secondary objectives:
1) Assessment of platelet aggregation in response to ADP and collagen at baseline and serially following tirofiban or cangrelor infusion.
2) Assessment of ADP-induced and thrombin induced platelet-fibrin clot strength by thrombelastography (TEG6S)
3) Assessment of thrombin generation kinetics by Calibrated Automated Thrombogram (CAT).

4) Real time evaluation of shear-induced thrombus formation using novel RUO T-TAS® *plus* system.
5) Assessment of High sensitivity troponin and CK-MB.

Study Type: This is a single center open label, randomized, observational study.

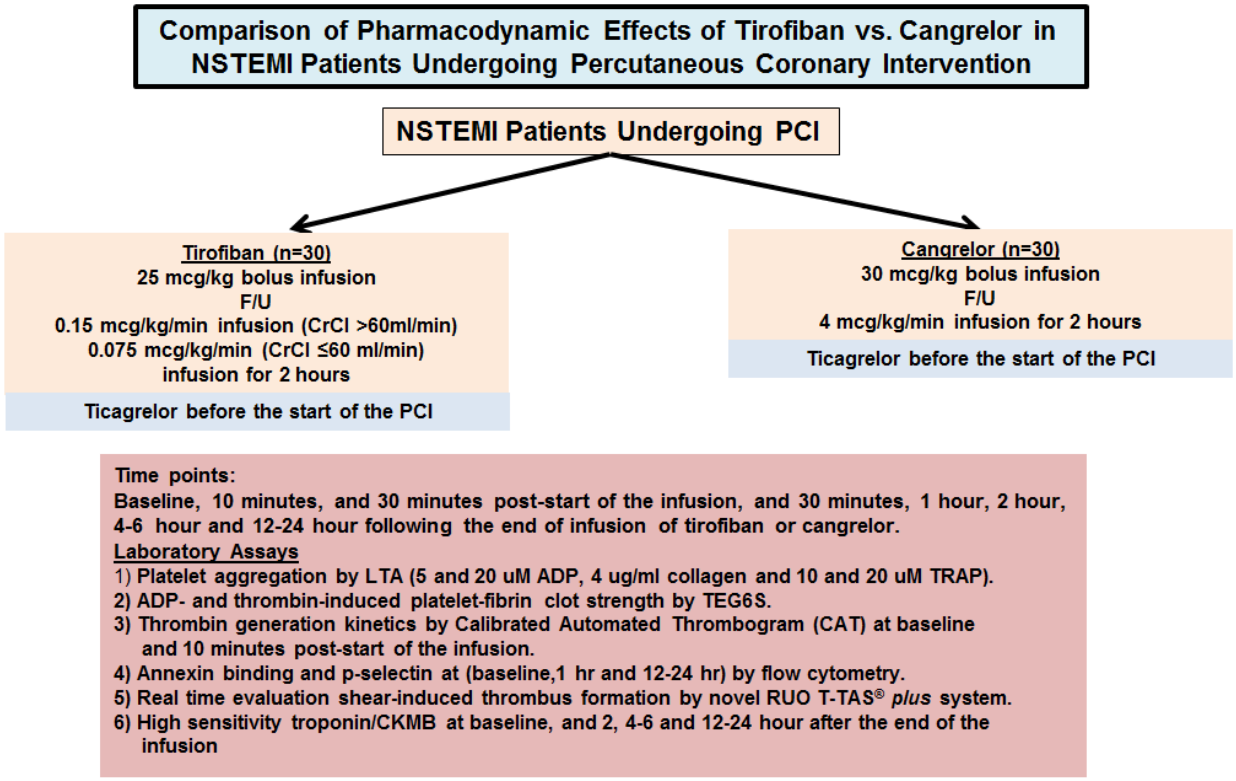
Study Design: Prospective, single center, observational study. NSTEMI patients undergoing PCI: 30 patients each will be treated with tirofiban or cangrelor (total n=60). The study is modelled after CLEAR PLATELETS study.[4]

Study Methodology: This investigation will be conducted in NSTEMI patients undergoing PCI at IHVI Fairfax hospital. Platelet activation and aggregation, clot characteristics, thrombin generation kinetics, real time thrombus formation, and high sensitivity troponin/CK-MB will be assessed at the baseline, and 10 minutes, 30 minutes post-start of the infusion and 30 minutes, 1 hour, 2 hour, 4-6 hour and 12-24 hour following the end of infusion of tirofiban or cangrelor (study flow diagram).

Patients: Sixty patients with NSTEMI undergoing PCI.

Statistical Methodology: Sample size (n=60 patients) is based on the CLEAR PLATELETS study.[4] Categorical variables will be compared using χ^2 test or the Fisher exact test as appropriate whereas continuous variables will be assessed using the Student unpaired t- or Mann-Whitney U test, and ANOVA test after checking for normal distribution. The pharmacodynamic effect of different regimens will be compared using a linear mixed model. Each model will include treatment, time, an interaction between treatment and time, and the baseline pharmacodynamic parameter as covariate. Analyses will be performed with SPSS software (SPSS, Inc., Chicago, IL) and $p<0.05$ will be considered significant.

Study Flow Diagram



1 INTRODUCTION

1.1 Specific Aims

To measure platelet activation and aggregation, clot characteristics, thrombin generation kinetics, real time thrombus formation at the baseline, and 10 minutes, 30 minutes post-start of the infusion and 30 minutes, 1 hour, 2 hour, 4-6 hour and 12-24 hour after the end of the infusion of tirofiban or cangrelor, and high sensitivity troponin and CK-MB at the baseline, 2 hour, 4-6 hour and 12-24 hour after the end of the infusion of tirofiban or cangrelor.

1) Platelet aggregation:
ADP-, collagen-, and TRAP -induced platelet aggregation by light transmittance aggregometry.

2) Platelet activation markers:
P-selectin, and annexin binding by flow cytometry.

3) Platelet-fibrin clot characteristics and ADP-induced platelet-fibrin clot strength:
Thrombin- and ADP-induced platelet-fibrin-clot strength, time to initial thrombin generation, and functional fibrinogen by thrombelastography (TEG6S).

4) Shear-induced platelet aggregation:
Shear-induced platelet aggregation by T-TAS perfusion flow chamber using novel RUO T-TAS® *plus* system.

5) Thrombin generation kinetics: thrombin generation kinetics by Calibrated Automated Thrombogram (CAT).

6) High Sensitivity troponin and CK-MB

1.2 Hypothesis

Serial evaluation of pharmacodynamic effects of tirofiban vs. cangrelor using various established laboratory assays will distinguish the superior pharmacodynamic benefits of tirofiban vs. cangrelor.

1.3 Background and Significance

It is estimated that, approximately 70% of the >780,000 persons with ACS in the United States, are diagnosed with NSTEMI.[1] Based on the CRUSADE (Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the ACC/AHA Guidelines) registry and ACTION Registry®-GWTG™ (Acute Coronary Treatment and Intervention Outcomes Network-Get With the Guidelines), 54-58% of NSTEMIs are managed with PCI.[5] Usually NSTEMI patients are older patients with more comorbidities, more risk for recurrent ischemic events and more incidences of previous MI.[1] Platelets play a critical role in periprocedural ischemic event occurrences including MI and stent thrombosis in patients with NSTEMI. Immediate potent inhibition of platelet function is critical for the prevention of periprocedural ischemic event occurrences in high risk N-ST segment elevation myocardial infraction (NSTEMI) patients undergoing percutaneous coronary intervention (PCI). Management of these patients during PCI with optimal antiplatelet therapy is crucial since these patients may require more effective antiplatelet therapy to overcome increased ischemic risk but bleeding risk is also high due to older age.

Currently, dual antiplatelet therapy with aspirin and an oral $P2Y_{12}$ receptor blocker (with loading doses) are widely used for PCI. However, immediate, potent and reversible inhibition

of platelet aggregation is not possible even with the newer oral agents, prasugrel and ticagrelor. Therefore, an intravenously administered GPIIb/IIIa receptor inhibitor (tirofiban) or $P2Y_{12}$ receptor blocker (cangrelor) with fast onset and offset of actions will provide more desired antiplatelet effects in the setting of PCI. Cangrelor is a parenterally administered adenosine triphosphate (ATP) analog with a short half-life (3–6min), with rapid onset/offset of action, and dose-dependent and predictable pharmacodynamic effects. Cangrelor provides effective inhibition of only ADP-mediated upstream platelet activation and aggregation pathways via selective inhibition of the $P2Y_{12}$ receptor. In the CHAMPION-PHOENIX trial, 11,145 patients with stable coronary artery disease (CAD) or acute coronary syndrome (ACS) received a bolus and infusion of cangrelor or a loading dose of 600 mg or 300 mg of clopidogrel. Cangrelor therapy was associated with a significantly reduced primary endpoint occurrence of death, MI, IDR, or stent thrombosis at 48 hours [4.7 vs. 5.9 %, odds ratio =0.78, p=0.005). The primary safety end point of severe bleeding at 48 hours was similar between the treatment groups [0.16% vs. 0.11% odds ratio=1.50, p=0.44]. The rate of stent thrombosis was lower in the cangrelor group compared with clopidogrel group [0.8% vs. 1.4%, odds ratio = 0.62, p=0.01]. Furthermore, the benefits associated with cangrelor were consistent across the subgroups of stable angina (n=1991), NSTEMI (n=2810), and STEMI (n=6138) (interaction, p=0.98), and whether the patient received clopidogrel 300mg LD or 600 mg LD (interaction, p=0.61). GUSTO severe bleeding was similar between groups.[6] Based on the favorable outcome observed in the CHAMPION-PHOENIX trial, the Food and Drug Administration Cardiovascular and Renal Drugs Advisory Committee recommended the approval cangrelor as an adjunct to PCI for reducing the risk of periprocedural thrombotic events such as MI, stent thrombosis and IDR.

A GPIIb/IIIa inhibitor such as tirofiban, will provide effective platelet inhibition by inhibiting the final common pathway of platelet aggregation stimulated by multiple agonists such as collagen, TRAP, and ADP. The latter may translate in to superior clinical benefits compared to cangrelor therapy.

2 STUDY DESIGN AND SUBJECT SELECTION

2.1 Study Type

This is a single center open label, randomized, observational study.

2.2 Setting

The study will be done at Inova Fairfax Hospital in an inpatient setting. Screening will take place at the emergency room (ER), coronary angiography preparation room, or coronary angiography suite. Study procedures after obtaining consent will occur at the emergency room and IHVI (coronary angiography suite, coronary angiography recovery room, intensive care coronary care unit, and coronary care step-down units).

2.3 Duration of Study

Subject participation will be until date of discharge.

2.4. Number of Subjects and Randomization procedure

Sixty patients with NSTEMI undergoing PCI will be randomized to receive 1:1 tirofiban or cangrelor. Allocation of study treatment will be performed via a web-based interactive randomization system, based on a computer-generated random sequence with a random block size.

2.5. Study Population

2.5.1. Population Characteristics

2.5.1.1. Gender and racial and ethnic origin of subjects

The study's intended population is inclusive of both genders (males and females), and all racial and ethnic groups and subgroups.

2.5.1.2. Age of Subjects

Subject enrollment will be comprised of subjects >18 years of age.

2.5.2 Vulnerable Population

Children, pregnant women, institutionalized persons, and persons with decisional incapacity will be not be enrolled in this study.

2.6. Recruitment

Recruitment will occur at the Inova Fairfax Hospital. The expected length of the recruitment period is 24 months. If the study conduct (e.g. recruitment rate; drop-out rate; data quality; protocol compliance) does not suggest a proper completion of the trial within the reasonable time frame as agreed upon, the recruitment period may be extended to reach the desired sample size.

2.7. Inclusion Criteria

The study will include subjects with non-ST elevation myocardial infraction (NSTEMI). Subjects may be enrolled if they appropriately fulfill all inclusion criteria.

1. NSTEMI meeting the following criteria:
   a) Patients 18 years of age or older with one or more of the following symptoms:
      -new ST-segment depression or transient elevation of at least 1 mm
      -elevations in troponin I, troponin T, or creatine kinase MB levels above ULN
   b) Eligible for ticagrelor, cangrelor, aspirin, UFH, and GP IIb/IIIa inhibitor treatment.
   c) Admitted at cardiac catheterization laboratory hospital or associated facility.
   d) Competent mental condition to provide informed consent.

2.8. Exclusion Criteria

Subjects will be excluded from study participation if any of the exclusion criteria is met.

1. Unstable angina with negative troponin, STEMI
2. Cardiogenic shock
3. Refractory ventricular arrhythmias
4. New York Heart Association class IV congestive heart failure
5. Cardiac arrest within 1 week of study entry

6. History of hemorrhagic or ischemic stroke, TIA, sub-arachnoid hemorrhage or intracranial neoplasm, arteriovenous malformation, or aneurysm
7. Fibrinolytic therapy within 48 hours of study entry
8. Active pathological bleeding or history of bleeding diathesis
9. Severe hepatic insufficiency
10. Current peptic ulceration
11. Increased bleeding risk, per investigator judgment
12. Known anemia (hematocrit<25%)/thrombocytopenia (platelet count < 100,000mm^3)
13. Surgery within 4 weeks before study entry or planned surgery within 2 months after study entry
14. Any $P2Y_{12}$ receptor inhibitor or GP IIb/IIIa inhibitor within 7 days of study entry
15. Receiving warfarin or other coumadin derivatives or NOACs within the last 10 days with an INR >1.5 secs or planned use during the hospitalization period
16. Contraindication to the use of ticagrelor and/or aspirin
17. Receiving or will receive oral anticoagulation or other oral antiplatelet therapy (except aspirin) that cannot be safely discontinued within the next 3 months
18. Receiving daily NSAIDs or COX2 inhibitors that cannot be discontinued or anticipated to require >2 weeks of daily NSAIDs or COX2 inhibitors during study
19. Investigational drug in last 30 days or presently enrolled in drug/device study
20. Pregnant or lactating women
21. Women of childbearing potential (post-menopausal women can be enrolled if at least 1 year of amenorrhea or surgically sterile) on oral contraceptives or planning a pregnancy.

    *Women of child-bearing potential who are not taking oral contraceptives and are not planning on becoming pregnant may be enrolled in the study. Safety urine pregnancy test will be done prior to and after the drug infusion.
22. Condition associated with poor treatment compliance (e.g., alcoholism, mental illness, or drug dependence)
23. Inability to provide written informed consent and to understand the full meaning of the informed consent

3 STUDY METHODS

3.1 Standard of Care Treatments

Subjects enrolled may be receiving a range of medical therapies as recommended by evidence-based guidelines. The investigator or qualified designee is responsible for verifying compliance in source documentation and ensuring that any other treatments delivered are according to the local standard of care. Standard of care treatments/parameters in the context of this study include:

1. Aspirin: 325 mg loading dose followed by 81 mg qd.
2. Ticagrelor: 180 mg loading dose administered in the catheterization laboratory before the start of the PCI followed by 90 mg bid.
3. Cangrelor: administered in the catheterization laboratory immediately prior to PCI- before guidewire is placed in the vessel (30 ug/kg bolus followed by 4ug/kg/min infusion for 2

hours)
4. Tirofiban: administered in the catheterization laboratory immediately prior to PCI- before guidewire is placed in the vessel
   (25 ug/kg bolus followed by 0.15 ug/kg/min (CrCl>60ml/min) or 0.075 ug/ml/min (CrCl≤60 ml/min infusion for 2 hours).
5. Unfractionated heparin (UFH) in tirofiban arm: as needed (e.g., 2,000 to 5,000 U) to achieve an ACT of 200 to 250 s.
6. Unfractionated heparin in the cangrelor arm: as needed to achieve an ACT of 250 to 300 s for HemoTec, 300 to 350 s for Hemochron

Subjects who were prescribed medications by his/her main provider that are prohibited during the study (see section 3.2) will be excluded or exited from the study.

3.2 Medications/Therapies Prohibited During the Study

The medications/therapies prohibited during the study are warfarin, anticoagulant other than UFH, oral direct anticoagulants, $P2Y_{12}$ receptor blockers other than ticagrelor or cangrelor, glycoprotein IIb/IIIa inhibitors other than tirofiban. Potential and enrolled subjects on or started on prohibited medications will be excluded or exited from the study.

3.3 Laboratory Measurements

Phlebotomy sites will be carefully chosen to minimize risk and platelet activation. After discarding the first 2-3 mL of free flowing blood, the blood collection tubes will be filled to capacity and gently inverted 3 to 5 times to ensure complete mixing of the anticoagulant. Tubes containing 3.2% trisodium citrate and hirudin will be used for flow cytometry, light transmittance aggregometry, thrombelastography, and shear-induced platelet aggregation.

- **Platelet Aggregation**

Platelet aggregation will be assessed in platelet-rich plasma using a Chronolog Lumi-Aggregometer (model 490-4D) with the AggroLink software package after stimulation with 5 and 20uM ADP, 4ug/ml collagen and 15 and 20uM TRAP).

- **Platelet Activation**

Expressions of p-selectin, and annexin binding will be assessed by flow cytometry using respective antibodies at baseline, and 1 hour and 12-24 hour after the end of the infusion**.**

- **Platelet-Fibrin Clot Characteristics**

Plate-fibrin clot characteristics will be assessed by the TEG6s instrument. TEG6S is a microfluidic fully automated cartridge-based device that provides quantitative and qualitative measurements of the physical properties of a clot. We will assess thrombin- and ADP-induced platelet-fibrin-clot strength, time to initial thrombin generation, and functional fibrinogen

- **Shear-Induced Platelet Aggregation**:

Shear-induced platelet aggregation by novel T-TAS® plus system.

- **Thrombin Generation Kinetics**:

Time to peak thrombin generation, peak thrombin generation, and total thrombin

generation kinetics will be assessed by Calibrated Automated Thrombogram (CAT). These parameters will be assessed at baseline and 10 minutes post-start of the infusion.

- **High Sensitivity Troponin and CK-MB**:

High sensitivity troponin and CK-MB will be assessed at baseline, and 2 hour, 4-6 hour and 12-24 hour after the end of the infusion.

3.4 Primary and Secondary endpoints

3.4.1 Primary Endpoint

Relative difference in 20 uM TRAP-induced platelet aggregation between cangrelor and tirofiban therapy at 30 minutes post-start of the infusion.

3.4.2 Secondary Endpoints

- Relative differences in 15 and 20 μM TRAP- induced platelet aggregation by light transmittance aggregometry between cangrelor and tirofiban therapy at all post-infusion time points except stated above.
- Relative differences in ADP and collagen-induced platelet aggregation by light transmittance aggregometry between cangrelor and tirofiban therapy at all post-infusion time points.
- Relative differences in P-selectin, and annexin binding between cangrelor and tirofiban therapy at 1 h and 12-24h after the end of the infusion.
- Relative differences in thrombin- and ADP-induced platelet-fibrin-clot strength, time to initial thrombin generation, and functional fibrinogen between cangrelor and tirofiban therapy at all post-infusion time points.
- Relative differences in shear-induced platelet aggregation between cangrelor and tirofiban therapy at all post-infusion time points.
- Relative differences in time to peak thrombin generation, peak thrombin generation and total thrombin generation between cangrelor and tirofiban therapy at 10 minutes post-infusion time point.
- Relative differences in peak high sensitivity troponin and CK-MB between cangrelor and tirofiban therapy will be assessed at 2 hour, 4-6 hour and 12-24 hour post-infusion time points.

3.4.3 Clinical Endpoints

Patients will be followed for clinical outcomes. All AEs and SAEs will be collected from randomization through 48 hours (+24 hours) after randomization. If there is a delay between time of randomization and start of study drug, AEs will be collected from time of randomization through 48 hours (+24) after study drug initiation. If a subject is discharged from the hospital prior to the 48 hour (+24 hour) assessment time-frame, subjects will receive a telephone call to review AEs and SAEs.

3.4.4. Adverse Events

Patients will be carefully monitored for AEs by the investigator during the designated study period. AEs and the treatment provided during the study should be recorded in the patient‘s treatment record and eCRF. Those events that are serious in nature will be reported to Medicure in an expedited manner. Patients experiencing AEs will be followed clinically until their health has returned to baseline status or until all parameters have returned to

normal or have otherwise been explained. The investigator will provide or arrange appropriate supportive care for the patient if necessary.

Adverse Event

An AE is defined as any untoward medical occurrence in a patient administered a medicinal product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of amedicinal product, whether or not considered related to the medicinal product.

Serious Adverse Event

A serious adverse event (SAE) is any untoward medical occurrence that:
Results in death,
Is life-threatening, i.e., the subject was, in the opinion of the investigator, at immediate risk of death from the event as it occurred .

Results in persistent or significant disability/incapacity,
Requires in-patient hospitalization or prolongs hospitalization,
Is a congenital anomaly/birth defect, or
Is another medically significant event that, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

Severity is an estimate or measure of the intensity of an AE, while the criteria for serious AEs are indications of adverse patient outcomes for regulatory reporting purposes. A severe AE need not necessarily be considered serious and a serious AE need not be considered severe. For example, nausea that persists for several hours may be considered severe nausea, but not an SAE. On the other hand, an MI that may be considered minor could also be an SAE if it prolonged hospitalization.

3.4.5. Bleeding

Bleeding will be assessed by the BARC scale. Bleeding will be assessed from the time of randomization through 48 hours after study drug initiation.

BARC criteria:

- Type 0: no evidence of bleeding.
- Type 1: bleeding that is not actionable and does not cause the patient to seek unscheduled performance of studies, hospitalization, or treatment by a healthcare professional. Examples include, but are not limited to, bruising, hematoma, nosebleeds, or hemorrhoidal bleeding for which the patient does not seek medical attention. Type 1 bleeding may include episodes that lead to discontinuation of medications by the patient because of bleeding without visiting a healthcare provider.
- Type 2: any clinically overt sign of hemorrhage (eg, more bleeding than would be expected for a clinical circumstance, including bleeding found by imaging alone) that is actionable but does not meet criteria for type 3, type 4 (CABG-related), or type 5 (fatal bleeding) BARC bleeding. The bleeding must require diagnostic studies, hospitalization, or treatment by a healthcare professional. In particular, the bleeding must meet at least

one of the following criteria: First, it requires intervention, defined as a healthcare professional–guided medical treatment or percutaneous intervention to stop or treat bleeding, including temporarily or permanently discontinuing a medication or study drug. Examples include, but are not limited to, coiling, compression, use of reversal agents (eg, vitamin K, protamine), local injections to reduce oozing, or a temporary/permanent cessation of antiplatelet, antithrombin, or fibrinolytic therapy. Second, the bleeding leads to hospitalization or an increased level of care, defined as leading to or prolonging hospitalization or transfer to a hospital unit capable of providing a higher level of care. Or third, the bleeding prompts evaluation, defined as leading to an unscheduled visit to a healthcare professional resulting in diagnostic testing (laboratory or imaging). Examples include, but are not limited to, hematocrit testing, hemoccult testing, endoscopy, colonoscopy, computed tomography scanning, or urinalysis. A visit or phone call to a healthcare professional during which neither testing nor treatment is undertaken does not constitute type 2 bleeding.

- Type 3: clinical, laboratory, and/or imaging evidence of bleeding with specific healthcare provider responses, as listed below:

- Bleeding Academic Research Consortium type 3a bleeding
  Any transfusion with overt bleeding

  Overt bleeding plus hemoglobin drop ≥3 to <5 g/dL (provided hemoglobin drop is related to bleeding). Hemoglobin drop should be corrected for intracurrent transfusion in which 1 U packed red blood cells or 1 U whole blood would be expected to increase hemoglobin by 1 g/dL.

- Bleeding Academic Research Consortium type 3b bleeding
  Overt bleeding plus hemoglobin drop ≥5 g/dL (provided hemoglobin drop is related to bleed). Hemoglobin drop should be corrected for intracurrent transfusion in which 1 U packed red blood cells or 1 U whole blood would be expected to increase hemoglobin by 1 g/dL.

  Cardiac tamponade
  Bleeding requiring surgical intervention for control (excluding dental/nasal/ skin/hemorrhoid)
  Bleeding requiring intravenous vasoactive drugs

- Bleeding Academic Research Consortium type 3c bleeding
  Intracranial hemorrhage (does not include microbleeds or hemorrhagic transformation; does include intraspinal); subcategories confirmed by autopsy, imaging, or lumbar puncture.
  Intraocular bleed compromising vision

- Type 4: Coronary Artery Bypass Graft–related bleeding
  Perioperative intracranial bleeding within 48 hours
  Reoperation after closure of sternotomy for the purpose of controlling bleeding

Transfusion of ≥5 U whole blood or packed red blood cells within a 48-hour period (only allogenic transfusions are considered transfusions for CABG-related bleeds)
Chest tube output ≥2 L within a 24-hour period

- Type 5: Fatal bleeding
  Fatal bleeding is bleeding that directly causes death with no other explainable cause. BARC fatal bleeding is categorized as either definite or probable as follows:

  - Probable fatal bleeding (type 5a) is bleeding that is clinically suspicious as the cause of death, but the bleeding is not directly observed and there is no autopsy or confirmatory imaging.
  - Definite fatal bleeding (type 5b) is bleeding that is directly observed (by either clinical specimen [blood, emesis, stool, etc] or imaging) or confirmed on autopsy.
  - The site of fatal bleeding is specified as intracranial, gastrointestinal, retroperitoneal, pulmonary, pericardial, genitourinary, or other.
  - Bleeding Academic Research Consortium fatal bleeding is meant to capture deaths that are directly due to bleeding with no other cause. The time interval from the bleeding event to the death should be considered with respect to likely causality, but there is no specific time limit proposed. Bleeding that is contributory but not directly causal to death is not classified as fatal bleeding but may be categorized as other forms of bleeding. Bleeding that leads to cessation of antithrombotic or other therapies may be contributory but again would not be classified as fatal bleeding. Bleeding associated with trauma or with surgery may be fatal, depending on whether it was determined to be directly causal or not.

    Examples of potential scenarios consistent with BARC fatal bleeding include the following: (1) A patient who receives a fibrinolytic agent for a small inferior MI loses consciousness and dies; autopsy shows an intracranial hemorrhage with mass effect: definite fatal bleed, intracranial; (2) a patient who receives a fibrinolytic agent for a large anterior MI loses consciousness and develops cardiac arrest; clinical examination immediately earlier showed a dilated left pupil: probable fatal bleed, intracranial; (3) a post-PCI patient on dual antiplatelet therapy who has a witnessed large gastrointestinal bleed becomes hypotensive and dies: definite fatal bleed, gastrointestinal; and (4) a patient develops a gastrointestinal bleed that is successfully cauterized; 3 days later, the gastroenterologist stops dual antiplatelet therapy and the patient has a fatal MI: not a fatal bleed.

3.5 Consent

Eligible subjects may only be included in the study after providing written (witnessed, where required by law or regulation), IRB -approved informed consent. If the subject is capable of doing so, he/she will indicate assent by personally signing and dating the written informed consent document. Informed consent must be obtained before conducting any study-specific procedures (i.e., all of the procedures described in the protocol). The process of obtaining informed consent will be documented in the subject source documents.

4 STATISTICAL CONSIDERATIONS/DATA ANALYSIS

4.1 Sample Size

This is an observational study. Based on CLEAR PLATELETS Study, we think 30 patients in each group are sufficient.

4.2 Statistical Calculations

Sample size (n=60 patients) is based on the CLEAR PLATELETS study. Categorical variables will be compared using χ^2 test or the Fisher exact test as appropriate whereas continuous variables will be assessed using the Student unpaired t or Mann-Whitney U test, and ANOVA test after checking for normal distribution. The pharmacodynamic effect of different regimens will be compared using a linear mixed model taking into account the within-subject correlation and a first-order autoregressive error structure. Each model will include treatment, time, an interaction between treatment and time, and the baseline pharmacodynamic parameter as covariate. Analyses will be performed with SPSS software (SPSS, Inc., Chicago, IL) and $p<0.05$ will be considered significant.

5 SCHEDULE OF PROCEDURES

The study comprises of 2 periods: Baseline/pre-study drug infusion and post-start of study drug infusion. Data analysis is summed up by timepoints: baseline/pre-study drug infusion, 10 minutes and 30 minutes post-start of the infusion and 30 minutes, 1 hour, 2 hour, 4-6 hour, and 12-24 hours post-end of study drug infusion.

5.1 Timing of Assessments

5.1.1 Baseline/Pre-study drug infusion

Subjects will be pre-screened to ensure that the subject is eligible for the study. During the baseline visit, the following will be completed and documented in the Case Report Forms (CRF):

- Obtain written informed consent
- Complete inclusion/exclusion criteria
- Obtain demographic information (i.e. date of birth, gender, and race)
- Record medical history (including medical, surgical, and smoking history)
- Record concomitant medications
- Obtain height and weight
- Obtain and record pre-intervention vital signs
- Obtain urine specimen for pregnancy test, if applicable
- Obtain blood specimen for platelet activation, aggregation, and biomarkers, clot strength and clot characteristics prior to study drug infusion (baseline)

5.1.2 Post-start of study drug infusion

Subjects are expected to be seen as an inpatient during the study. Time-points for this period include 10 minutes and 30 minutes post-start of the infusion and 30 minutes, 1 hour, 2 hour, 4-6 hour, and 12-24 hour post-end of study drug infusion (a 10% window at each time point is acceptable). During these time points, blood specimens for platelet activation, aggregation, clot characteristics, and biomarkers, will be obtained. If applicable, urine

specimen for pregnancy test will be obtained at 12-24 hours post-end of drug infusion. Continued eligibility, changes in concomitant medications, and subject status will be collected.

6 DATA MANAGEMENT

6.1 Data Storage and Management

Designated study site staff will record data required by the protocol into the CRFs and enter it into the electronic database. Authorized research staff will review the CRFs for completeness and accuracy and make any necessary corrections to the data entered into the electronic database. Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Each subject screened and enrolled will be assigned a subject identification number (ID) and a list of subjects with their corresponding subject ID will be maintained separately from collected data. Physical CRFs will be stored in the research site in a locked office and electronic subject data will be locked in a password protected file on a secure internet server, accessed only by authorized research staff.

6.2 Records Retention

The Investigator will maintain the records of final CRFs (CDROM copies), worksheets, paper source documents, and all other study-specific documentation in accordance with ICH Guidelines. Essential documents should be retained until at least three (3) years after the investigation is formally discontinued.

6.3 Confidentiality

Subject information will be kept confidential as according to HIPAA requirements. Subject data will be stored and managed as outlined in section 6.1. All data records will be stored on site until 2 years after the investigation is formally discontinued. Paper records will be shredded and recycled. Records stored on a computer hard drive will be erased using a commercial software application designed to remove all data from the storage device.

7 DATA SAFETY MONITORING PLAN

7.1 The principal investigator holds ultimate responsibility for the oversight and execution of the data and safety monitoring plan. This research site will be fully committed in the ongoing review and refinement of the trial's processes to assure subject safety, data validity and integrity, and regulatory compliance.

7.2 The principal investigator and an independent medical monitor will review the safety data, assure protocol compliance, and conduct safety reviews to protect each subject's safety and welfare in this clinical investigation. The review process will be initiated after enrolling the first 10 subjects and every 10 subjects thereafter. The principal investigator will evaluate whether the study should continue unchanged, require modification, or closed the enrollment.

7.3 Case report forms (CRF) will be completed to capture protocol data. Two study coordinators on site will do separate CRF audits to assure data is accurate and complete and to assure adherence to study protocol. This will allow for identification of protocol deviations and areas in which practice can be improved. Audits will be done on all paper CRFs, which include the subject's informed consent form, ICF notes, subject eligibility,

medical records, concomitant medication list, laboratory results, study procedures, visit notes, investigator notes, and adverse event forms. Proper filing of required regulatory documentation and subject CRFs, correct transcription of data, which will include checking for appropriate units of measure and legibility, will be monitored.

8 HUMAN SUBJECTS PROTECTION (RISKS, BENEFITS, AND ALTERNATIVES)

8.1 Risks

8.1.1 Potential loss of privacy

Protected health information (PHI) will be collected during the study. The risk for breach of confidentiality and privacy will be minimized by shielding the subjects unlinking his or her identity from his or her personal health information.

8.1.2 Adverse events

All adverse events will be collected and reported. SAEs will be treated appropriately and managed as according to standard of care.

8.1.3 Economic risk

No economic risk is expected.

8.2 Benefits and Alternatives

Participation in the study is entirely voluntary. The alternative is not to participate in the trial. Patients may benefit from the antithrombotic properties of the parenteral agents.

9 SUBJECT COMPENSATION

9.1 Costs

The subject or their insurance company will not be billed for this study. All study related tests that are not considered as standard of care will be paid for by the research site.

10 ADVERSE EVENT REPORTING

10.1 Adverse Event Reporting

The principal investigator has the primary responsibility for SAE identification, documentation, grading, and assignment of attribution to the study intervention. SAEs related to study procedures will be collected and reported (see section 6.1.2). SAEs must be recorded in the AE CRF with the following information:

-The intensity grade (see CTCAE v4.03 grading)
-The relationship to study procedure
-Attribution
-Duration
-Occurrence (known risks for study procedure-expected, unexpected)
-Other contributing causes
-Actions in response to event
-Outcome
-Criteria for SAE

10.2 Protocol Deviations and Violations

The principal investigator will not deviate from the protocol without obtaining approval from the IRB or Ethics Committee and the sponsor. Protocol deviations that occur will be reported to the Inova IRB at the time of continuing review. Protocol violations that affect the subject's

rights and safety, and/or affects study integrity will be reported to the Inova IRB within 10 working days of event knowledge.

11 FUNDING

This research is funded by Medicure Pharma, Inc. There is no investigational product involved. Treatment assigned to the subject will be as according to main provider's discretion as according to standard of care.

12 CONFLICTS OF INTEREST

Dr. Gurbel reports personal fees from AstraZeneca, Boehringer Ingelheim, Merck, Janssen Pharmaceuticals, Bayer, and Haemonetics; grants from Haemonetics, Merck, Harvard Clinical Research Institute, National Institutes of Health, Coramed Technologies, MedImmune, a patent for platelet function testing; and stock options in Merck.

13 FACILITIES AND EQUIPMENT

The research site is equipped with its own laboratory equipment, which includes state of the art technologies for platelet assays, centrifuges, refrigerators, and freezers for study specimen processing and storage.

14 OUTSIDE CONSULTANTS/COLLABORATORS

There are no outside consultants/collaborators participating.

15 CONTRACTURAL AGREEMENTS

There are no outside consultants/collaborators participating.

16 REFERENCES

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