Influence of Vorapaxar on Thrombin Generation and Coagulability

Principal Investigator: Paul Gurbel, MD

Sub-Investigator(s):

Sponsor: MERCK & Co., Inc.

Site of Investigation:
Inova Heart and Vascular Institute at Inova Fairfax Hospital, VA

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Title: Influence of Vorapaxar on Thrombin Generation and Coagulability

Short Title: Vorapaxar and Thrombin Generation

Rationale: PAR-1 receptor inhibition is an emerging therapeutic strategy in patients who have suffered ACS. Vorapaxar is a novel antiplatelet agent that selectively inhibits the cellular actions of thrombin through antagonism of PAR-1. Currently, there are no data available regarding the effect of vorapaxar on clot generation kinetics or TIP-FCS when added to standard of care antiplatelet regimens. Potential reduction of TIP-FCS and clot generation kinetics by vorapaxar may assist in our understanding of the mechanism of action and in personalizing therapy in high risk patients to effectively reduce recurrent thrombotic event occurrences.

Objectives: To determine onset-, maintenance-, and offset-effect of vorapaxar on thrombin generation kinetics (clotting time, thrombin generation, platelet-fibrin clot strength, and clot lysis) in antiplatelet naïve patients and patients on mono and dual antiplatelet therapy.

Study Type: Experimental study design

Study Design: This phase IV, prospective cohort (4 groups), non-randomized, open label, pharmacodynamics, and safety investigation.

Study Methodology: This investigation will be conducted in patients 18-75 years of age with multiple coronary artery disease risk factors (antiplatelet naïve patients) and patients with prior MI or PAD on antiplatelet therapy. Pharmacodynamics will be assessed at multiple time points to assess onset-, maintenance-, and offset-effect of vorapaxar on thrombin generation, platelet reactivity, and plasma/platelet endothelial and inflammatory biomarkers. Safety assessment will be assessed throughout the study.

The cohorts include:

Subjects with Multiple Risk Factors:

Group 1: Subjects with multiple risk factors and antiplatelet naïve (n=25).

Group 2: Subjects with multiple risk factors and antiplatelet naïve. 75mg QD clopidogrel QD for ≥ 7 days (n=25).

Subjects with Prior MI or PAD:

Group 3: Subjects with 81mg QD Enteric Coated (EC) Acetylsalicylic Acid (ASA) (n=25).

Group 4: Subjects with 81mg QD EC ASA+75mg QD clopidogrel (n=25).

Statistical Methodology: A very brief description of main elements of the statistical methodology to be used.

1 INTRODUCTION

1.1 Specific Aims

- To determine onset-, maintenance-, and offset-effect of vorapaxar on thrombin generation kinetics (clotting time, thrombin generation, platelet-fibrin clot strength, and clot lysis) in antiplatelet naïve patients and patients on mono and dual antiplatelet therapy.
- To determine onset-, maintenance-, and offset-effect of vorapaxar on platelet reactivity induced by ADP, collagen, arachidonic acid, and SFLLRN.
- To determine the effect of vorapaxar on plasma platelet/endothelial and inflammatory biomarkers in antiplatelet naïve patients and patients on mono and dual antiplatelet therapy.

Hypothesis

- Vorapaxar will reduce TIP-FCS, clot generation kinetics, and plasma biomarkers associated with haemostasis and inflammation in patients on standard of care antiplatelet regimens.
- Vorapaxar effects will be more marked in patients with high TIP-FCS, a risk factor for post-PCI ischemic events.

1.2 Background and Significance

The occurrence of coronary arterial thrombotic events during acute coronary syndromes (ACS) and percutaneous coronary interventions (PCI) are critically dependent on reactive platelets. Antiplatelet therapy plays a central role in preventing stent thrombosis and recurrent myocardial infarction in these high risk patients. Platelet activation involves multiple signaling pathways activated by thrombin, thromboxane A_2, adenosine diphosphate (ADP) and collagen that interact with specific receptors. Simultaneous and optimal blockade of these pathways is essential to ensure effective inhibition of platelet function and attenuation of thrombotic events. However, it remains unclear which pathway is central to the generation of thrombotic events in an individual patient. Emerging data suggests that activation of the protease-activated receptors (PARs) by thrombin and platelet-dependent thrombin generation may be patient specific. Evidence for this concept is present in the significant residual risk (~10%) present in high risk patients treated with potent $P2Y_{12}$ blockers and ASA.

Translational antiplatelet therapy studies thus far have largely focused on the measurement of platelet aggregation and agglutination to fibrinogen-coated beads in anticoagulated blood. These studies ignore the characteristics of platelet-fibrin clot formation as a potential contributor to the development of adverse events (i.e. no study of platelet-fibrin interactions). In addition to platelet function, thrombin mediated platelet-fibrin clot characteristics may play an important role in the development of ischemic events and stent restenosis. In support of this hypothesis, the POST-STENTING study clearly demonstrated that studying platelet function in isolation may have an important limitation in predicting ischemic events as well as determining effective strategies to reduce recurrent adverse events. In the latter study, we found that high platelet reactivity was a comparatively poor indicator of ischemic events following stenting, relative to measurements of platelet-fibrin clot characteristics.

Adverse events were more or less equally distributed in the middle two quartiles of post-treatment platelet aggregation. Most importantly, thrombin-induced maximum platelet-clot strength (TIP-FCS) measured at discharge was the most powerful predictor of 6 months post-stenting ischemic events with a sensitivity of 74%, specificity of 89%, and odds ratio 22.6 (1^{st} quartile vs. 4^{th} quartile). In the

latter study, 74% of patients with ischemic events had high TIP-FCS (>72 mm, upper quartile value). These results indicate that platelet-fibrin clot strength may play important roles in the development of adverse ischemic events. Those subjects who form the most robust platelet-fibrin clots carry the greatest risk for recurrent thrombotic event occurrence. Of critical importance, these results also indicate that current long term antiplatelet therapies are inadequate to reduce adverse events in selected patients. Novel, longer-term treatment strategies directed at reducing thrombin function in selected patients may have significant impact in reducing adverse events.

Thrombin potently activates platelets through the protease-activated receptor (PAR-1). PAR-1 receptor inhibition is an emerging therapeutic strategy in patients who have suffered ACS. Vorapaxar is a novel antiplatelet agent that selectively inhibits the cellular actions of thrombin through antagonism of PAR-1. In the TRA 2P trial, in patients with a history of heart attack or with peripheral arterial disease (PAD) who had no history of stroke or transient ischemic attack (TIA), vorapaxar added to standard of care was associated with a significant 17 percent relative risk reduction over the three years in the combined events of cardiovascular (CV) death, myocardial infarction (MI), stroke, and urgent coronary revascularization (UCR) [event rate 10.1 percent vs. 11.8 percent for placebo]. For the key secondary composite efficacy endpoint of CV death, MI, and stroke alone, vorapaxar produced a significant 20 percent relative risk reduction in these patients [7.9 percent vs. 9.5 percent for placebo]. Based on these results, the U.S. Food and Drug Administration recently approved Zontivity (vorapaxar) to reduce the risk of heart attack, stroke, and cardiovascular death for secondary prevention in patients with a history of MI and peripheral vascular disease.

Currently, there are no data available regarding the effect of vorapaxar on clot generation kinetics or TIP-FCS when added to standard of care antiplatelet regimens. Potential reduction of TIP-FCS and clot generation kinetics by vorapaxar may assist in our understanding of the mechanism of action and in personalizing therapy in high risk patients to effectively reduce recurrent thrombotic event occurrences.

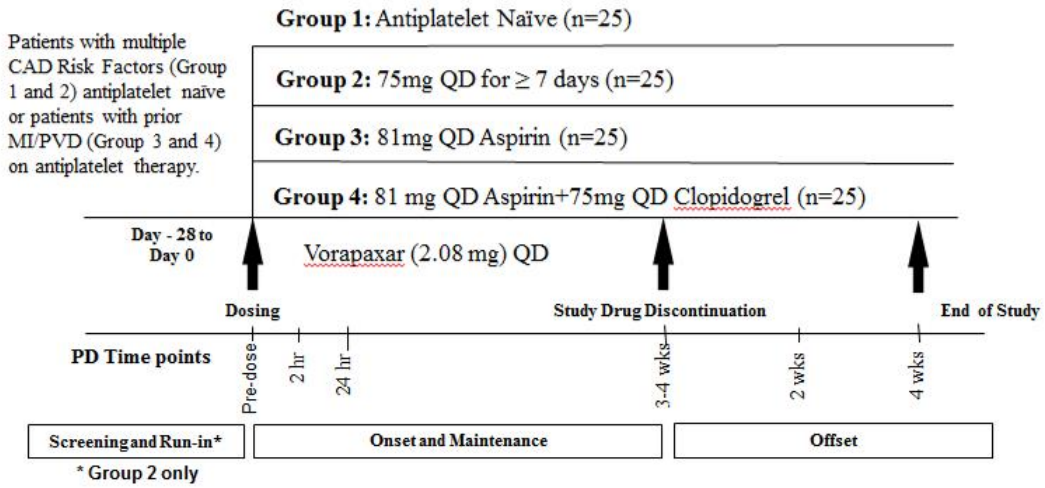
2 STUDY DESIGN AND SUBJECT SELECTION

2.1 Study Type

This phase IV, open label, pharmacodynamic investigation will be conducted in patients 18-75 years of age with multiple coronary artery disease risk factors (antiplatelet naïve patients) and patients with prior MI or PAD on antiplatelet therapy (Figure 1). All Subjects will be enrolled at Inova Fairfax Medical Center. Enrollment is expected to be completed within 6-8 months after first individual is enrolled. Informed consent will be obtained from subjects meeting the inclusion criteria before the initiation of any study-specific procedures. Pharmacodynamics will be assessed at multiple time points to assess onset-, maintenance-, and offset-effect of vorapaxar on thrombin generation, platelet reactivity, and plasma/platelet endothelial and inflammatory biomarkers. Safety assessment will be assessed throughout the study.

Figure 1

Influence of Voropaxar on Thrombin Generation and Coagulability



PD Measurements

- Thrombin generation kinetics by TEG-6S and Thrombovision
- 5uM ADP-, 15 uM SFLLRN (PAR-1) and 4ug/ml collagen-induced aggregation by LTA and TEG-6S
- Plasma platelet/endothelial and inflammatory biomarkers

Safety Assessments

- Safety labs and physical assessment at screening, 24 hrs, 3-4 weeks, and at end of study
- SAE and AE collection

2.2 Number of Subjects

Multiple cohorts of subjects may be utilized in order to achieve the target enrollment number of 100 subjects (25 qualified subjects in each study group) with valuable data.

2.3 Study Population

2.3.1 Population Characteristics

2.3.1.1 Demographics

Demographic data will be collected at the screening visit. These data will include:

- Gender
- Date of birth
- Race/ ethnicity

2.3.1.2 Medical history

Medical history which includes previous diagnoses, diseases, surgeries, smoking history and concomitant medications will be collected.

2.3.2 Gender of Subjects

The study's intended population is inclusive of both sexes/genders (males and females), and all racial and ethnic groups and subgroups. Female subjects are eligible for participation in the study only if they are of non-childbearing potential (i.e., physiologically incapable of becoming pregnant).

2.3.3 Age of Subjects

Subject enrollment will be comprised of men and women between 18 and 75 years of age with history of myocardial infarction and/or peripheral artery disease, or who have ≥ 2 risks factors of developing cardiovascular disease (type 2 diabetes, obesity, current smoking, hypertension, hypercholesterolemia, history of a thrombotic event while on ASA therapy).

2.3.4 Vulnerable Populations

Vulnerable population will not be included as it is not appropriate for the scientific goals of the research proposed.

2.4 Recruitment

Recruitment will occur at the Inova Heart and Vascular Institute. The length of the recruitment period is 8.5 months. Multiple strategies will be employed to identify potentially eligible subjects for this study. These will include (but are not restricted to) database screening, chart screening, and patient screening at physician offices and at internal medicine, cardiology, and vascular disease/imaging clinics.

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.

2.5 Inclusion Criteria

The study will include subjects who have multiple risk factors of developing atherosclerosis or evidence or a history of atherosclerosis involving the coronary or peripheral vascular systems. Subjects may be enrolled if they appropriately fulfill all inclusion criteria and none of the exclusion criteria.

Inclusion Criteria: Subjects must meet ALL the criteria listed below for entry:

1. Subject may be of either sex and of any race, and must be between 18 and 75 years of age.
2. Subject must have multiple risk factors of developing atherosclerosis, or evidence of a history of atherosclerosis involving the coronary or peripheral arterial systems as follows:
   a. Subject must present with multiple risk factors for CAD or PAD such as high blood pressure, high cholesterol, diabetes, obesity, current smokers, 55 years or older, or
   b. CAD as indicated by a history of presumed spontaneous MI (hospitalized with final diagnosis of MI, excluding periprocedural or definite secondary MI due to profound anemia or hypertensive emergency or troponin increase in sepsis) at least 1 month prior to enrollment, or
   c. PAD as indicated by a history of intermittent claudication and
      i. a resting ankle/brachial index (ABI) of <0.85, or

ii. significant peripheral artery stenosis (>50%) documented by angiography or non-invasive testing by duplex ultrasound, or
iii. previous limb or foot amputation for arterial vascular disease (excludes trauma), or
iv. previous aorto-femoral bypass surgery, limb bypass surgery or percutaneous transluminal angioplasty of the iliac or infrainguinal arteries.

3. Subject must be willing and able to give appropriate informed consent.
4. The subject is able to read and has signed and dated the informed consent document including authorization permitting release of personal health information approved by the investigator's Institutional Review Board (IRB).

2.6 Exclusion Criteria

Exclusion Criteria: Subjects will be excluded from entry if ANY of the criteria listed below are met:

1. Clinically unstable at the time of enrollment.
2. Any planned coronary revascularization or peripheral intervention.
3. Concurrent or anticipated treatment with warfarin (or derivatives, e.g., phenprocoumon), oral factor Xa inhibitor, or oral direct thrombin inhibitor after enrollment.
4. Concurrent or anticipated treatment with a potent inducer (e.g., rifampin) or potent inhibitor (e.g., ketoconazole, erythromycin) of CYP3A4 isoenzymes (see Appendix B) (but see note in text for exceptions).
5. History of a bleeding, or evidence of active abnormal bleeding.
6. History at any time of intracranial hemorrhage, intracranial or spinal cord surgery, or a central nervous system tumor or aneurysm.
7. Documented sustained severe hypertension (systolic blood pressure >200 mmHg or diastolic blood pressure >110 mmHg) at enrollment or within the previous 10 days.
8. Severe valvular heart disease, as defined by the American College of Cardiology /American Heart Association.
9. History within 30 days before enrollment of invasive surgeries (other than mentioned above), or is anticipating one during the course of their study participation, or is planning to have one within 1 month post dosing with the study drug.
10. History within 30 days before enrollment of TIA and ischemic (presumed thrombotic) stroke/CVA.
11. Known platelet count <100,000/mm3 within 30 days before enrollment.
12. Known active hepatobiliary disease, or known unexplained persistent increase in serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) activity to two times or more the upper limit of the reference range (upper limit of "normal" [↑2xULN]).
13. Any serious illness or any condition that the investigator feels would (a) pose a significant hazard to the subject if investigational therapy were initiated, or (b) would limit the prognosis of the subject, regardless of investigational therapy.
14. Any serious medical comorbidity (e.g., active malignancy) such that the subject's life expectancy is <24 months.
15. Known current substance abuse at the time of enrollment.
16. Current participation in any other study of investigational therapy or participation in such a study within the last 30 days.
17. Known hypersensitivity to any component of the current investigational product.
18. Female subject of child-bearing potential.

19. Subject is a woman who is breast-feeding.
20. Subject is part of the staff personnel directly involved with this study or is a family member of the investigational staff.

3 Treatments

3.1 Treatments to Be Administered

The study drugs to be administered in this trial include the: PAR1 antagonist, vorapaxar; enteric coated (EC) Acetylsalicylic Acid **(**ASA); and P2y12 inhibitor, clopidogrel.

3.1.1 Run-in

Eligible subjects (excluding subjects in group 1) who have signed informed consent will complete the run-in period. Subjects will be instructed to continue on their prescribed antiplatelet regimen, EC ASA and/or clopidogrel; however, the medications will be provided to the subjects by the study site for compliance accounting purposes. Study diaries will be provided for the run-in period only (7 -10 days) and pill reconciliation will be performed for all visits. The subjects will postpone taking their antiplatelet regimen in order to take the dose during this visit from the medication provided by the study site. Subjects will be assigned into the following groups:

- Subjects in group 2 will receive clopidogrel 75mg orally daily provided by the study site. Subjects in group 3 will receive EC ASA 81 mg orally daily provided by the study site.
- Subjects in group 4 will receive clopidogrel 75mg in addition to EC ASA 81mg orally daily provided by the study site.

Subjects will be grouped according to their current antiplatelet regimen. Subjects with cardiovascular risk factors not on antiplatelet therapy will be included in either Group 1 or 2. Patients on ASA alone will be included in Group 3 and patients on dual antiplatelet therapy will be in Group 4. All doses will be in tablet form for oral administration.

3.1.2 Vorapaxar Treatment

Subjects that have received a confirmation of laboratory results and inclusion criteria in the antiplatelet naïve (group 1) group and those who have completed the run-in period with adherence to treatment (no more than 2 doses missed) with EC ASA and/or clopidogrel and who wish to continue in the study will receive vorapaxar 2.08mg daily for 30 ± 7 days. Subjects will be instructed with regards to any interactions the study drugs may have with other medications the subject is taking or may have at home. This will be in addition to the standard treatment assigned to each group as shown below:

- Group 1: vorapaxar 2.08mg orally once daily
- Group 2: clopidogrel 75mg orally once daily + vorapaxar 2.08mg orally once daily
- Group 3: EC aspirin 81mg orally once daily + vorapaxar 2.08mg orally once daily
- Group 4: EC aspirin 81mg orally once daily + clopidogrel 75mg orally once daily + vorapaxar 2.08mg orally once daily

3.1.3 Identity of Study Treatment

All study drugs will be labeled in the local language according to the requirements of local law and legislation. A complete record of batch numbers and expiry dates of all study treatment as well as the labels will be maintained in the site's study file.

3.2 Dosage and Administration

Vorapaxar 2.08mg will be taken orally once daily with or without food.

Subjects in group 2 will receive 75 mg orally daily with or without food.

Enteric coated (EC) aspirin 81 mg will be taken orally once daily. EC aspirin should not be crushed or chewed and is to be taken with a full glass (8oz) of water with or without food.

All study treatment will be generally taken in the morning. Subjects who missed a study treatment dose need to take the missed dose as soon as they remember. Skip the missed dose if it is almost time for the next scheduled dose. Do not take an extra dose to make up for the missed dose.

Assigned study drug treatments for each group are specified in section 3.1.1 and 3.1.2.

3.3 Dose Modifications

The investigator should permanently stop the study drug for a given subject if continuation is deemed to be detrimental to the subject's well-being. Permanent study drug interruption should be recorded on the corresponding case report form giving the date and primary reason for the permanent discontinuation of study drug.

3.4 Drug Logistics and Accountability

All study drugs will be stored at the investigational site in accordance with GCP and Good Manufacturing Practices (GMP) requirements. Tablets will be stored as according to the manufacturer's instructions and will be inaccessible to unauthorized personnel. Special storage conditions and a complete record of batch numbers and expiry dates can be found in the sponsor's study file. The responsible site personnel will confirm the condition, date and time of receipt of study drug and will use the study drug only within the framework of the clinical study protocol. Receipt, distribution, return, and destruction of study drugs will be documented and filed as according to the site's standard operating procedures.

3.5 Treatment Compliance

The investigator should promote compliance by counseling the subjects to take the study drug as prescribed. The subjects will be instructed to contact the appropriate site personnel if unable to comply with study drug instructions. Treatment compliance will be assessed by reviewing a study drug diary during the run-in period and by mandatory study drug pill count on visits 2, 3, and 4. Study drug compliance should be documented on the appropriate case report form.

3.6 Prior and Concomitant Therapy

Vorapaxar increases the risk of bleeding in proportion to the subject's underlying bleeding risk and use of certain concomitant medications (chronic nonsteroidal anti-inflammatory drugs [NSAIDS], selective serotonin reuptake inhibitors, serotonin norepinephrine reuptake inhibitors) can increase the risk for bleeding. Careful consideration of each potential subject's overall health status, medical history, and concomitant medications will be given by the investigator after a thorough review to ensure subject's appropriateness for enrollment.

Vorapaxar is eliminated primarily by metabolism with contributions fromCYP3A and CYP2J2. Strong CYP3A inhibitors increase and inducers decrease vorapaxar exposure.

Subjects who are taking CYP3A inhibitors, CYP3A inducers, warfarin and other anticoagulants, antiplatelet medications except for clopidogrel, oral factor Xa inhibitor, or oral direct thrombin inhibitor and subjects who are planning to start treatment on these medications are not eligible for enrollment.

4 STUDY METHODS

4.1 Reactive Hyperemia-Peripheral Arterial Tonometry (RH-PAT)

Noninvasive flow-mediated vasodilation (FMD) is a widely used method to assess endothelial function, but its technical difficulty and problems remain obstacles for use in clinical practice. Reactive hyperemia-peripheral arterial tonometry (RH-PAT) was developed as a simpler and more reproducible method. Endothelial function will be assessed using the ENDOPAT system (Itamar Medical Ltd) at pre-dose (Visit 2) and after 3-4 weeks of maintenance therapy (Visit 4).

4.2 Blood and Urine Sampling

Phlebotomy sites will be carefully chosen to minimize risk and the blood will be collected into Vacutainer® tubes (Becton-Dickinson, Franklin Lakes, NJ). After discarding the first 2-3mL of free flowing blood, the 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 (n=2) will be used for light transmission aggregation, Calibrated Automated Thrombogram and for platelet/ endothelial and inflammatory biomarker assays. One 17 USP/mL lithium heparin tube will be used for TEG-6S PlateletMapping assay.

4.3 CORA TEG-6S

The CORA instrument is a microfluidic cartridge-based device capable of performing all current thromboelastographic assays. It is a fully automated cartridge-based system, replacing the cups and pins of the TEG5000 and eliminating potential user error in pipetting and reagent constitution. Unmetered samples are transferred into the cartridge using a disposable dropper or transfer pipette. There are up to four simultaneous channels per cartridge, and the system requires less than 70ìl of blood for one channel, and less than 280ìl for four channels, versus 360ìl for one TEG channel. To perform a test, a disposable CORA Cartridge is inserted into the instrument. Blood or WQC material is added to an entry port on the cartridge and drawn into the cartridge under instrument control. Once in the disposable, the sample is automatically metered into as many as four separate analysis channels, depending upon the assay being performed. Reconstitution of reagents dried within the cartridge is accomplished by moving the sample through reagent wells, under the control of microfluidic valves and bellows within the cartridge. After each sample has been mixed with reagent, Cora Hemostasis Analyzer System (Cora®) will be used to assess qualitative and quantitative assessment of the hemostasis. Two cartridges will be used in the study.

Citrated Multi-Channel cartridge is used to measure hemostasis including thrombin generation kinetics (Table). The assay cartridge uses a citrated blood sample that is mixed with dried reagents within each of the 4 channels, each with calcium chloride (to reverse the sodium citrate) and : 1)

kaolin, 2) kaolin + tissue factor activated (RapidTEG), 3) kaolin + heparinase, and 4) kaolin + abciximab (functional fibrinogen).

R	Period of time of latency from the time that the blood was placed in the TEG® analyzer until the initial fibrin formation. This represents the enzymatic portion of coagulation.
K	K time is a measure of the speed to reach a certain level of clot strength. This represents clot kinetics.
α	Measures the rapidity of fibrin build-up and cross-linking (clot strengthening). This represents fibrinogen level.
MA	Maximum Amplitude is a direct function of the maximum dynamic properties of fibrin and platelet bonding via GPIIb/IIIa and represents the ultimate strength of the fibrin clot. This represents platelet function/aggregation.
TMRTG	Time to maximum rate of thrombus generation.
MRTG	Maximum rate of thrombus generation.
TG	Total thrombus generated.
TMRL	Time to maximum rate of lysis.
MRL	Maximum rate of lysis.
L	Total lysis.
D	Delta is the difference between R time and the time of initial split point (SP, mins) of the TEG tracing (R – SP), representing the time interval of greatest clot growth secondary to peak thrombin generation.

The PlateletMapping (PM) cartridge is used to monitor response to oral antiplatelet agent including ASA and plavix. The cartridge employs a heparinized blood sample that is mixed with dried reagents within each of the 4 channels of the cartridge: 1) kaolin + heparinase, 2) Activator F + abxicimab, 3) ADP + Activator F, and 4) AA + Activator F.

4.4 Calibrated Automated Thrombogram® (CAT) System:

Calibrated Automated Thrombogram® **(**CAT) System: Lag time, peak thrombin production, mean velocity rate index, and endogenous thrombin potential (ETP) will be assessed by calibrated automated thrombogram in platelet poor plasma (Thrombinoscope by Stago).

4.5 Platelet aggregation (Chronolog)

The blood-citrate tubes will be centrifuged at 120g for approximately 5 minutes to recover platelet-rich plasma and will be further centrifuged at 850g for approximately 10 minutes to recover platelet-poor plasma. The platelet rich plasma and platelet-poor plasma will be stored at room temperature to be used within 2 hours of blood collection.

Platelet aggregation will be assessed using Chronolog Lumi-Aggregometer (model 490-4D) with the AggroLink software package. Platelets in platelet rich plasma will be stimulated with 5µM ADP-, 15µM SFLLRN(PAR-1) -and 4µg/ml collagen- induced maximal and final aggregation values (aggregation at 6 minutes) will be recorded with platelet-poor plasma used as a reference.

4.6 Plasma Platelet/Endothelial and Inflammatory Biomarkers

After collection plasma and serum samples will be batched and frozen at -70C at each time point. Plasma platelet/endothelial and inflammatory biomarkers measurements to be performed in the study include high sensitivity C-reactive protein (hsCRP), fibrinogen, von Willebrand factor (vWF), interleukin (IL)-6, p-selectin, plasminogen activator inhibitor (PAI)-1, matrix metalloproteinase (MMP)-9).

Serum samples will be batched and shipped on dry ice for analysis by Corgenix Medical Corp. (Broomfield, CO) for measurements of oxidized LDL-â2 glycoprotein1 complex, a pro-atherothrombotic antigen.

4.7 CYP2C19 Buccal Swab Genotyping

CYP2C19 Buccal Swab Genotyping Study subjects considered for clopidogrel groups (Group 2 and 4) will have a CYP2C19 buccal swab analysis performed at screening. Poor cytochrome P450 2C19 metabolizers (*2/*2) will be a screen failure and discontinue the study.

The Spartan RX CYP2C19 System is indicated for use as an aid to clinicians in determining therapeutic strategies for therapeutics that are metabolized by the Cytochrome P450 2C19 gene product, and that are specifically affected by the *2, *3, and *17 alleles. The Spartan RX CYP2C19 Assay will be run on the Spartan RX CYP2C19 Platform from the buccal sample collected with a buccal swab.

4.8 Study Treatment/Intervention-

This phase IV, open label, pharmacodynamic investigation will be conducted in patients between 18-75 years of age with multiple coronary artery disease risk factors (antiplatelet naïve patients) and patients with prior MI or PAD on antiplatelet therapy (Figure 1). Informed consent will be obtained from subjects meeting the inclusion criteria before the initiation of any study-specific procedures. Subjects will be grouped according to their current antiplatelet regimen. Subjects with cardiovascular risk factors not on antiplatelet therapy will be included in either Group 1 or 2. Patients on ASA alone will be included in Group 3 and patients on dual antiplatelet therapy will be in Group 4.

- Group 1 (control group): vorapaxar 2.08mg orally once daily
- Group 2: clopidogrel 75mg orally once daily + vorapaxar 2.08mg orally once daily
- Group 3: EC aspirin 81mg orally once daily + vorapaxar 2.08mg orally once daily
- Group 4: EC aspirin 81mg orally once daily + clopidogrel 75mg orally once daily + vorapaxar 2.08mg orally once daily

The study comprises 6 periods: screening, run-in (for subjects in groups 2, 3, and 4), onset and maintenance, offset, and end of study (Figure 1). The trial will require clinic visits at screening, vorapaxar dosing day, 24 hours post initial vorapaxar dosing, 3-4 weeks post-dosing, 2 weeks post vorapaxar discontinuation, and 4 weeks post vorapaxar discontinuation. Patient instructions for the run-in phase will be given during the screening visit. One day prior to the run-in, the study coordinator will call the subject to remind subjects of run-in instructions.

Pharmacodynamics will be assessed at multiple time points to assess onset-, maintenance-, and offset-effect of vorapaxar on thrombin generation, platelet reactivity, and plasma/platelet endothelial and inflammatory biomarkers. Safety assessment will be assessed throughout the study. Approximately 25-30ml of blood will be drawn at each visit. Phlebotomy sites will be carefully chosen to minimize risk. See schedule of procedures in section 7 for detailed schedule of study events.

4.9 Consent

Eligible subjects may only be included in the study after providing written (witnessed, where required by law or regulation), IRB/IEC/REB-approved informed consent, If the subject is capable of doing so, he/she should indicate assent by personally signing and dating the written informed consent document or a separate assent form. 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 should be documented in the subject source documents.

5 STATISTICAL CONSIDERATIONS/DATA ANALYSIS

5.1 Sample Size

This is an exploratory study; we assume that a sample size of 25 qualified subjects (see study schematic) in each group is sufficient to assess the difference in platelet inhibitory effect of vorapaxar 2.08 mg, administered alone or in addition to an antiplatelet agent (clopidogrel 75mg), and/or acetylsalicylic acid 81mg.

5.2 Primary and Secondary Analysis

5.2.1 Primary Analysis

The primary endpoint measures are a change in platelet-fibrin clot generation kinetics by thrombelastography (R, TIP-FCS, TG) and thrombin generation kinetics (Lag time, peak thrombin production, time to peak thrombin generation, and endogenous thrombin potential) as measured by calibrated automated thrombogram at maintenance therapy (3-4 weeks vorapaxar treatment) compared to pre-dose within and between each treatment group.

5.2.2 Secondary Analysis

Secondary Analysis endpoint measures are a change in:

- Platelet-fibrin clot generation kinetics by thrombelastography (R, TIP-FCS, TG) at onset and offset vorapaxar treatment compared to pre-dose within and between each treatment group.
- Thrombin generation kinetics (Lag time, peak thrombin production, time to peak thrombin generation, and endogenous thrombin potential) as measured by calibrated automated thrombogram during onset and offset of vorapaxar treatment compared to pre-dose within and between each treatment group.
- 5µM ADP-, 15µM SFLLRN (TRAP)-and 4µg/ml collagen- induced maximal and final aggregation by light transmittance aggregation during onset, maintenance, and offset of vorapaxar treatment compared to pre-dose within and between each treatment group. ADP-and

AA-induced aggregation by TEG-6S Platelet Mapping assay during onset, maintenance, and offset of vorapaxar treatment compared to pre-dose within and between each treatment group.

- Platelet/endothelial and inflammatory biomarkers during onset, maintenance, and offset of vorapaxar treatment compared to pre-dose within and between each treatment group. Reactive hyperemia index (RHI) as assessed using the ENDOPAT system between pre-dose and maintenance therapy within and between the treatment groups.

6 Statistical calculations

Statistical calculations will be carried out using SAS® for Windows, version 9.3 or later (Cary, NC). Within group analyses of change scores (pre and post vorapaxar) will be performed using a paired t-test, or the Wilcoxon signed ranks test if the change scores are sufficiently non-normal. The Shapiro-Wilk test will be used to determine if the change scores are non-normal. A calculated P-value < 0.05 will be considered a significant difference between treatments. A secondary ANCOVA analysis will be performed in each group to examine the factors that explain potential variations in change scores, and model variables will include the baseline test value, age, sex, race, weight, diabetes, antiplatelet response to EC ASA and clopidogrel, smoking, etc.

7 DATA MANAGEMENT

7.1 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.

7.2 Records Retention

The Investigator will maintain the records of drug disposition, final CRFs (CDROM copies), worksheets, source documents, and all other study-specific documentation in accordance with ICH Guidelines. Essential documents should be retained until at least two (2) years after the investigation is formally discontinued.

7.3 Confidentiality

Subject information will be kept confidential as according to HIPAA requirements. Subject data will be stored and managed as outlined in section 5.3. 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.

8 DATA SAFETY MONITORING PLAN

8.1 Oversight

8.1.1 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.

8.1.2 The principal investigator and medical monitor will monitor 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 to enrollment. Dr. Palak Shah will serve as medical monitor and review all study related adverse events.

8.1.3 Case report forms (CRF) will be completed to capture protocol data. Two study coordinators 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, adverse event forms, subject drug diary, study drug accountability, and visit schedule. 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.

8.2 Criteria for protocol review

Safety related criteria will be in place for continued dosing of subjects. If any of the criteria, listed below, are met, all available data will be reviewed by the principal investigator to provide a recommendation regarding subsequent enrollment.

- A serious adverse event (SAE), deemed related to study treatment, occurs.
- Any subject on treatment experiences bleeding (major or spontaneous).
- Any subject on treatment experiences a Common Terminology Criteria for Adverse Events (CTCAE; v4.03) Grade 3 (or higher) event, deemed related to study treatment.
- Any other event that occurs in a subject on active treatment that is deemed to pose an unacceptable risk to other subjects in the study.

8.3 Subject Safety

8.3.1 Informed Consent

The informed consent form provides important safety information so each subject can make an informed decision on whether to participate in the trial. Subjects will be encouraged to complete the trial, but can withdraw at any time. By giving consent, health information, safety laboratory tests, vital signs measurements, and medical records will be collected and reviewed.

8.3.2 Physical Exam

A physical exam will be conducted by the investigators during the screening period after obtaining consent to evaluate the general status of the subject and to further elucidate patient symptoms, risk factors, or concerns that may increase the subject's risk for adverse reactions to the study treatment. Physical examinations will also be conducted by the investigators 30 $\pm$ 7 days post the first study drug dose.

8.3.3 Adverse Events Collection

Identification of adverse events is important in monitoring for patient safety. Adverse events will be collected during each study visit using a combination of modalities which include, spontaneous reporting of AEs by the patient in response to an open question from study personnel, observation, physical examination, or other diagnostic procedures performed during the study. All subjects will be instructed to report any new or worsening signs and symptoms or clinical events. Adverse events will be reported and assessed for clinical significance by the investigator. Unscheduled clinic visits, additional safety laboratory tests, and other diagnostic procedures may be necessary as recommended by the investigator to evaluate subject safety and to further investigate AEs reported by subjects in-between scheduled clinic visits.

8.3.4 Clinic Visits

Clinic visits will be scheduled as follows: 24 hours (+/- 2 hours) post initial dose of study drug, 30 $\pm$ 7 days post initial dose of study drug, 44 days post initial dose of study drug, and end of study visit. Clinic visits will allow for subject safety observations through patient assessment, review of subject's continued eligibility, safety laboratory test collection (for visit 4), vital signs measurement, physical examination (by investigators for visit 4), and recording of changes in concomitant medications and AEs.

8.3.5 Patient Counseling

All subjects will be counseled and given instructions in regards to study treatments. Subject education will include study treatment action, side effects, benefits and risks, dosage and administration, food-drug/drug-drug interactions, pregnancy/lactation warning, and when to call investigator/physician. Subjects will be instructed to inform physicians and dentists of study treatment intake and to list all prescription medications, over-the-counter medications, or dietary supplements they are taking or plan to take so study personnel knows about other treatments that may affect adverse event risk.

8.4 Study Treatment Discontinuation

Study treatment will be stopped and subject removed from study participation if any of the following criteria listed below are met:

- Withdrawal of informed consent
- Meeting an exclusion criteria post screening
- Any serious adverse event (as defined in section 8.8.2)
- Major or spontaneous bleeding
- Adverse events as defined by CTCAE v4.03, grade 3 or higher
- Allergic reaction to study treatment

- Pregnancy
- Subject requires an invasive or surgical procedure within the study period
- Non-compliance
- Subject develops a need for anticoagulant therapy
- Subject develops a need for antiplatelet therapy not originally assigned in his/her respective group
- Subject develops a need for any medication in the exclusion list
- Any other reason that the investigator feels would pose a significant hazard to the subject if investigational therapy were continued

8.5 Subject Withdrawal

Subjects should also be withdrawn at any time if the investigator concludes that it would be in the subject's best interest for any reason.

Subjects may be considered withdrawn if they fail to return for visits, or become lost to follow up for any other reason. For subjects who are lost to follow-up (i.e., those subjects whose status is unclear because they fail to appear for study visits without stating an intention to withdraw), the investigator should show "due diligence" by documenting in the source documents steps taken to contact the subject (e.g. dates of telephone calls, registered letters). Subjects who are prematurely withdrawn from the study will not be replaced.

8.5.1 Subject Withdrawal Documentation.

If premature withdrawal occurs for any reason, the investigator should attempt to determine the primary reason for the withdrawal. The date and primary reason for premature withdrawal will be recorded on the Study Summary CRF. If applicable, the Investigator must provide appropriate follow-up medical care for all subjects who are prematurely withdrawn from the study, or must refer them for appropriate ongoing care.

8.6 Human Subjects Protection (Risks, Benefits, and Alternatives)

8.6.1 Risks

8.6.1.1 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.

8.6.1.2 Potential Adverse Events

The subject's medical history, significant laboratory and diagnostic tests, and concomitant medications will be thoroughly reviewed prior to enrollment to reduce incidence of adverse events.

8.6.1.3 Study Drug

8.6.1.3.1 Bleeding

GUSTO severe bleeding was defined as fatal, intracranial, or bleeding with hemodynamic compromise requiring intervention; GUSTO moderate bleeding was defined as bleeding

requiring transfusion of whole blood or packed red blood cells without hemodynamic compromise. (GUSTO: Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Arteries.). Vorapaxar increased GUSTO moderate or severe bleeding by 55%. It increases the risk of bleeding, including intracranial hemorrhage (ICH) and fatal bleeding. Note that the risk of bleeding with vorapaxar increases in proportion to the subject's underlying bleeding risk. Provide treatment if subject shows signs and symptoms of bleeding.

Thienopyridines, including clopidgorel, increase the risk of minor and major bleeding. In the CURE trial, Plavix use with ASA was associated with an increase in major bleeding (primarily gastrointestinal and at puncture sites) compared to placebo with ASA. Gastrointestinal hemorrhage occurred at a rate of 2% in those taking clopidogrel vs. 2.7% in those taking ASA in the CAPRIE trial. Other bleeding events that were reported more frequently in the clopidgorel group were epistaxis and hematoma.

8.6.1.3.2 Thrombotic Thrombocytopenic Purpura (TTP)

TTP has been reported following use of clopidogrel. It is characterized by thrombocytopenia, microangiopathic hemolytic anemia, neurological findings, renal dysfunction, and fever.

8.6.1.3.3 Non-Hemorrhagic Adverse Reactions

In the TRA 2°P / TRA•CER study, non-hemorrhagic adverse reactions specified below occurred at least 2% in the vorapaxar group and at least 10% greater than placebo.

- Anemia
- Depression
- Rashes, eruptions, and exanthemas

8.6.1.3.4 Iron Deficiency and Oculomotor Disturbances

The following adverse reactions below occurred at a rate less than 2% in the vorapaxar group but at least 40% greater than placebo in descending order:

- Iron deficiency
- Retinopathy or retinal disorder
- Diplopia/oculomotor distrubances

An increased rate of diplopia and related oculomotor disturbances was observed with vorapaxar treatment (30 subjects, 0.02% vs. placebo (10 subjects, 0.06%). While some cases resolved during continued treatment, information on resolution of symptoms was not available for some cases.

8.6.1.3.5 Allergic Reactions

Subjects may experience an allergic response to vorapaxar/clopidogrel or any its component. Discontinue study treatment if signs and symptoms of an allergic reaction are noted.

8.6.1.3.6 Pain, discomfort, or bruising

Pain, discomfort, or bruising from phlebotomy sites may occur related to the blood sampling process. Fainting and a decrease in blood pressure can happen although rare.

There is also a very small risk that a nerve could be damaged during insertion of a needle. Phlebotomy sites will be carefully chosen to minimize risk.

8.6.1.3.7 Mild discomfort in the arm from a pressure cuff

Subjects may experience mild discomfort in the arm from a pressure cuff while inflated as related to blood pressure measurement and Endo-PAT2000 test.

8.6.1.3.8 Local irritation

Local irritation from ECG electrodes may occur. Some hair may need to be clipped in small patches to accurately measure an ECG. Removal of electrodes may cause local irritation and rash.

8.6.1.3.9 Other potential adverse events

Other potential adverse events as related to subject's concomitant diseases. To prevent subjects from missing any medications while in-clinic, all subjects will be instructed to take all approved concomitant medications (i.e., insulin, anti-hypertensive medications) with them to be taken as allowed by study protocol. A small snack will also be provided to subjects after laboratory and endo-pat testing to prevent hypoglycemic episodes, especially for diabetic patients. Water will be allowed throughout each outpatient visits.

8.6.2 Economic risk

Subjects in the study may lose time at work or home and spend more time in the research site more than usual. Visit schedules will be made flexible for subjects (as allowed by protocol).

8.7 Benefits and Alternatives

There are no direct benefits to the patient. This study can help healthcare providers in understanding the mechanism of action of vorapaxar when added to aspirin and/or clopidogrel. Participation in the study is entirely voluntary. The alternative is not to participate in the trial.

8.8 ADVERSE EVENTS

8.8.1 Adverse Event (AE) Definitions

An AE is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product which does not necessarily have to have a causal relationship with this treatment. An adverse event can be any unfavorable and unintended sign (e.g., including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality (i.e., whether or not it is considered to be drug-related). This includes any newly occurring event or previous condition that has increased in severity or frequency since the administration of the study treatment/intervention.

A suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

An adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event. An adverse event or suspected adverse reaction is considered "unexpected" if it is not

specifically mentioned as occurring with the particular drug under investigation. Information about common side effects already known about the study drug/s drug can be found in the study drug/s package inserts. This information will be included in the subject's informed consent and should be discussed with the subject during the study as needed.

8.8.2 Serious Adverse Event (SAE) Definition

An SAE is defined as an event that:

- is fatal or life-threatening;
- results in persistent or significant disability/incapacity;
- constitutes a congenital anomaly/birth defect;
- requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
  - routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
  - elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study drug
  - treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
  - social reasons and respite care in the absence of any deterioration in the subject's general condition
- is medically significant, i.e., defined as an event that jeopardizes the subject or may require medical or surgical intervention to prevent one of the outcomes listed above

8.8.3 AE Grading Scale

The descriptions and grading scales found in NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be used for AE reporting. All appropriate treating areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded (http://ctep.cancer.gov). This version of CTCAE is MedDRA v12.0 (Medical Dictionary for Regulatory Activities Terminology) compatible at the AE term level where each CTCAE term is a MedDRA LLT (Lowest Level Term). Each AE term is associated with a 5-point severity scale. Bleeding will be documented using the Bleeding Academic Research Consortium (BARC) standard definition (Appendix A) (Mehran et al., 2011).

8.8.4 Procedures for Recording and Reporting of Adverse Events

8.8.4.1 All AEs will be reported to the principal investigator and medical monitor for evaluation. For both serious and non-serious AEs, the investigator has the primary responsibility for AE identification, documentation, grading, and assignment of attribution to the study treatment/intervention. The sponsor will consider the investigator's view when assessing the safety of the drug and determining whether to report expeditiously to the FDA and other regulatory agencies.

8.8.4.2 AEs occurring from the start of study medication administration through the last day of study participation must be recorded on the AE CRF with the following information:

- The intensity grade (grade 1, 2, 3, 4, 5; see CTCAE v4.03 grading)
- The relationship to the study drug(s)

- Attribution: An assessment of the relationship between the AE and the medical intervention (i.e., study drug administration). After naming and grading the event, the clinical investigator must assign an attribution to the AE using the following attribution categories:

RELATIONSHIP	ATTRIBUTION	DESCRIPTION
Unrelated to study treatment/intervention	Unrelated	The AE *is clearly NOT related* to the study treatment/intervention
	Unlikely	The AE *Is doubtfully related* to the study treatment/intervention
Related to study treatment/intervention	Possible	The AE *may be related* to the study treatment/intervention
	Probable	The AE *is likely related* to the study treatment/intervention
	Definite	The AE *is clearly related* to the study treatment/intervention

- The duration (start and end dates or if continuing at final exam)
- Occurrence (known risks for study drug/s, underlying illness or population)
  - Expected
  - Unexpected
- Other contributing causes
- Action taken with study drug
- Any other actions in response to event
- Outcome
  - Death related to AE
  - Recovered/resolved with sequelae
  - Not recovered/resolved
  - Recovered/resolved without sequelae
  - Recovering/resolving
  - Intervention for AE continues
  - Unknown
- Whether it constitutes a serious adverse event (SAE)

8.8.5 AE Collection

The occurrence of adverse events should be sought by non-directive questioning of the subject at each visit during the study. AEs may also be detected when these are volunteered by the subject during or between visits or through physical examination, laboratory test, or other assessments. Medical conditions/diseases present before starting the study drug are considered AEs only if they worsen after starting the study drug. Abnormal laboratory values or test results constitute AEs only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy. Abnormal values that constitute a SAE or lead to discontinuation of administration of study drug must be reported and recorded as an AE. Adverse event collection will commence when the subject starts taking study medication.

8.8.6 Adverse Event Treatment

All AEs should be treated appropriately and managed as according to standard of care, at the discretion of the investigator. The action taken to treat the AE should be recorded on the AE CRF. A detected AE should be followed until its resolution or until the subject completes the study. Assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, relationship to the study drug, the interventions required to treat it, and the outcome.

8.8.6.1 Treatment for Bleeding Events

Bleeding will be documented using the Bleeding Academic Research Consortium (BARC) standard definition (Appendix A) (Mehran et al., 2011). Bleeding events should be treated according to standard clinical practice, depending upon the location and severity of the bleed. Withholding vorapaxar for a brief period will not be useful in managing an acute bleeding event because of its long half-life. There is no known treatment to reverse the antiplatelet effect of vorapaxar.

8.8.6.2 Treatment for Allergic Reactions

Subjects will be observed for signs and symptoms of an allergic reaction while taking study drug/s and managed with appropriate therapy. The investigator may use his discretion in determining the regimen to be used according to standard clinical practice and tailored to the specific clinical situation (i.e., symptoms and severity of the reaction and responsiveness). Any occurrence of allergy signs and symptoms is cause for discontinuation of study treatment/s.

8.8.7 AE Grading Scale

Severity is a measure of intensity where seriousness is defined by the criteria listed below. An AE of severe intensity may not always 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. AEs which are not serious but which lead to permanent discontinuation of study medication will be captured in the CRF, but do not require expedited reporting. Non-serious AEs which do not lead to discontinuation of study medication will not be collected.

8.8.8 SAE Reporting

To ensure subject safety, every SAE, regardless of suspected causality, occurring after the subject signs informed consent and until 4 weeks (28 days) after the subject has stopped study participation must be reported to the principal investigator within 24 hours of learning of its occurrence. Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode, regardless of when the event occurs. An SAE that is considered completely unrelated to a previously reported one should be reported separately as a new event. Information about all SAEs is collected and recorded on the Serious Adverse Event Report Form. The investigator must assess the relationship to study drug and complete the SAE Report. The original copy of the SAE Report Form must be kept with the case report form documentation at the study site.

The follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the subject continued or withdrew from study participation.

If the SAE is not previously documented in study drug/s Package Insert (new occurrence) and is thought to be related to the Zontivity (vorapaxar), the investigator may urgently require further information from the investigator for Health Authority reporting. MERCK or designee may need

to issue an IND Safety Letter (Investigator Notification) to inform all investigators involved in any study with the same drug that this SAE has been reported.

The investigator is responsible for promptly notifying the institutional review board (IRB) of all SAEs, including any significant follow-up information. In agreeing to the provisions of this protocol, the investigator accepts the legal responsibilities for prompt notification of any SAE.

8.8.9 Protocol Deviations

The principal investigator will not deviate from the protocol without obtaining approval from the IRB or Ethics Committee and the sponsor. In medical emergencies, the investigator will use medical judgment and will remove the subject from immediate hazard, then notify the sponsor, and the IRB or Ethics Committee immediately regarding the type of emergency and course of action taken. Any action in this regard will be recorded on the appropriate CRF.

9 SCHEDULE OF PROCEDURES

The study comprises of 6 periods: screening, run-in (for subjects in groups 2, 3, and 4), onset and maintenance, offset, and end of study. The trial will require clinic visits at screening, vorapaxar dosing day, 24 hours post initial vorapaxar dosing, 3-4 weeks post-dosing, 2 weeks post vorapaxar discontinuation, and 4 weeks post vorapaxar discontinuation. Patient instructions for the run-in phase will be given during the screening visit. One day prior to the run-in, the study coordinator will call the subject to remind subjects of run-in instructions.

A tabulated overview (Table 1*)* of the procedures conducted in each of these periods is provided in section 9.1 and the procedures and their timing are described in more detail in the remaining sections.

9.1 Tabulated overview

Table 1. Schedule of evaluations

	V1		V2	V3	V4	V5	V6/EOS
	Screening (28 days from V2)	**Run-In* (Start 7 ± 3 days prior to scheduled V2)**	**Group 1: 3-28 days of screening Groups 2,3,4: 7-28 days of Screening**	**24 hours of 1st study drug dose**	**30 +/- 7 days 1st study drug dose**	**14 +/- 3 days of Visit 4**	**14 +/- 3 days of Visit 5**
Informed Consent	X						
Inclusion/Exclusion Criteria	X						
Medical History	X						
Review Prior/Concomitant Medications	X	X#	X	X	X	X	X
Vital Signs	X		X	X	X	X	X
12 lead ECG	X						
Blood and Urine for Safety Labs (CBC,CMP,UA,hsCRP)	X=				X		X
Physical Examination	X				X		
Endo-PAT2000 Test			X		X		
PD/hsCRP/urine			X	X	X	X	X
Buccal Swab for CYP2C19*2 Testing	X						
Dispense Study Drug		X#	X		X#	X#	
Administer Vorapaxar			X				
ASA and/Clopidogrel Dairy Completion		X#	X				
Drug accountability			X	X	X	X#	X#
Adverse Events		X#	X	X	X	X	X

X Applies to all subjects for that visit
X# Applies only to subjects in groups 2, 3, and 4
X= Blood and Urine Safety Labs collection *only* for subjects with no lab results on record that are ≤ 30 days from screening visit
Screening and run-in visit can occur at the same day if the subject's medical records and safety laboratory results (should be ≤30 days from screening) are readily available for review. For these subjects, instructions should be given the day of screening/run-in visit to hold any standard home treatment of EC ASA and/or clopidogrel (if assigned to groups 2, 3, and 4). Schedule the screening/run-in visit 7+3 days from visit 2.

9.2 Timing of Assessments

9.2.1 Screening

Subjects will be contacted prior to screening visit to ensure that the subject is eligible for the study. The screening visit will take place within 28 days (to allow time for medical records and safety laboratory results collection and review) from the vorapaxar dosing day (visit 2).

Subjects with medical records and laboratory results (within 30 days), readily available for review, can undergo screening and run-in phase at the same visit (only if assigned to groups 2, 3, and 4). If this is the case, schedule the screening/run-in visit 7+3 days from visit 2. Subjects with a scheduled screening/run-in visit will be instructed by the study coordinator not to take their EC ASA and/or clopidogrel at home in the morning of scheduled visit. Subject will be given study EC ASA and/or clopidogrel at the research site. If medical records and lab results are not available at the screening visit, patients will be asked to return to site for run-in phase.

During the screening 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 history and smoking history)
- Record medication history
- Perform complete physical examination
- Obtain height and weight
- Obtain and record vital signs
- Buccal cell sample will be collected for CYP2C19*2 testing. The actual date and clock time of buccal sampling will be recorded in the CRF.
- Obtain clinical safety laboratory specimens/results comprehensive metabolic panel, complete blood count with platelets and auto differential, high sensitivity C-reactive protein) *Note: Laboratory results collected within 30 days of screening are allowed for review.*
- Obtain urine samples for urine analysis and urine thromboxane. *Note: Urinalysis results collected within 30 days of screening are allowed for review.*
- Obtain 12-lead ECG *Note: EKG results collected within 90 days of screening are allowed for review.*
- Provide patient education about medication intake:
  - Continue standard home treatment for EC aspirin and clopidogrel until next study visit (run-in or visit 2).
  - Subjects who are currently using any medications not approved by the Investigator will be excluded. .
  - Avoid grapefruit or grapefruit juice during the study period.
  - If the subject's medical records and laboratory results are readily available for a thorough review by the investigator during the screening visit (i.e., able to access

medical records and laboratory results in INOVA EPIC system), the run-in phase can be completed at the same visit (see section 9.2.2)

- Schedule next visit. Once verified to be eligible for enrollment, subjects in group 1 will be scheduled for visit 2 and 3. Subjects belonging in groups 2, 3, and 4 will be scheduled for the run-in phase, visit 2, and 3. Subjects will be informed via phone call to confirm scheduled visits.

9.2.2 Run-In Phase

Subjects in groups 2, 3, and 4 will undergo a run-in phase once deemed eligible for enrollment. The run-in phase will be started 7 + 3 days prior to a scheduled visit 2 (vorapaxar dosing day). Subjects will be informed via phone call, a day before, to hold their current home medication of clopidogrel and/or EC ASA the morning of scheduled run-in visit.

If the subject has medical records and safety laboratory test results readily available for review during the screening visit, the run-in phase can be completed at the same time as screening. Instruct subjects to hold home medication of clopidogrel and/or EC ASA the morning of scheduled screening/run-in visit. During this visit, the following procedures will be performed:

- Collection of adverse events.
- Collection of concomitant medications.
- Confirm continue eligibility.
- Administer first dose of study treatment. Document first dose of study treatment as specified below as day 1 of the run-in phase.
  - Group 2: Administer one dose of clopidogrel 75mg orally
  - Group 3: Administer one dose of EC ASA 81mg orally.
  - Group 4: Administer one dose of clopidogrel 75mg and EC ASA 81mg.
- Dispense study drug (EC ASA and/or clopidogrel) for subjects in groups 2, 3, and 4 only.
  - Group 2: 10 tablets of clopidogrel 75mg Group 3: 10 tablets of EC ASA 81mg
  - Group 4: 10 tablets of clopidogrel 75 mg and EC ASA 81mg
- Provide patient education (for EC ASA and/or clopidogrel).
- Provide subject instructions.
  To continue on their prescribed antiplatelet regimen, clopidogrel and/or EC ASA; however, the medications will be provided to the subjects by the study site with the following instructions::
  - Group 2 subjects: Take clopidogrel 75mg orally once a day starting the next day, preferably in the morning.
  - Group 3 subjects: Take EC ASA 81mg orally once a day starting the next day, preferably in the morning.
  - Group 4 subjects: Take clopidogrel 75mg and EC ASA 81mg orally once a day starting the next day, preferably in the morning.
- Provide study drug diary for all subjects. Document first dose of study treatment as day 1 of the run-in phase.

9.2.3 Visit 2

The study coordinator will call the eligible subjects the day before to review protocol instructions and study related activities for Visit 2 and answer any potential questions. Remind the subjects to hold their clopidogrel and/or EC ASA (study medications)and all other medications taken by the subjects regularly. Subjects will be instructed to bring all home medications with them for the appointment to take after the bloodwork and testing. Ask subjects to avoid any high fat foods the morning of the visit.. For the Endo-PAT2000 test, the subject needs to abstain from smoking and caffeine 2 hours prior to testing. Subjects also need to hold any vasoactive medications 24 hours prior to the test.

The dosing visit will occur within 3 to 28 days of screening for subjects in group 1. Visit 2 for groups 2, 3, and 4 will occur within 7 + 3 days from the run-in period.

During this visit, the following procedures will be performed:

- Vital signs (blood pressure, heart rate) will be obtained after the subject has been in a sitting position for at least 5 minutes.
- Collection of Study Drug Diary for subjects in groups 2, 3, and 4.
- Study drug accountability will be performed (groups 2, 3, and 4).
- Administer Endo-PAT2000 test.
- Blood and urine samples will be obtained for baseline PD measurements through direct venipuncture. The actual date and clock time of blood/urine sampling will be recorded in the CRF.
- Provide a small snack after blood and urine specimen collection
- If applicable, after lab work collection and endo-PAT2000test, remind subject to take approved home medications as according to subject's regular schedule. Subjects will receive first dose of vorapaxar 2.08mg orally. After administering first dose of vorapaxar, administer daily dose of study treatment (clopidogrel and/or EC ASA) to subjects as according to assigned group (to prevent a missing dose).
- Subject will return to clinic in 2 hours (+ 60 mins) post vorapaxar dosing time for PD blood draw.
- Collection of adverse events.
- Collection of concomitant medications.
- Dispense study drugs
    - Group 1: A bottle of 37 tablets of vorapaxar 2.08mg
    - Group 2: A bottle of 37 tablets of vorapaxar 2.08mg and 37 tablets of clopidogrel 75mg
    - Group 3: A bottle of 37 tablets of vorapaxar 2.08mg and 37 tablets of EC ASA 81mg.
    - Group 4: A bottle of 37 tablets of vorapaxar 2.08mg, 37 tablets EC ASA 81mg, and 37 tablets of clopidogrel 75mg.
- Provide patient education for vorapaxar
- Remind subject of visit 3 schedule and protocol instructions, which includes avoiding any high fat foods for breakfast, and to hold vorapaxar, clopidogrel and/or EC ASA the morning of visit 3. Instruct subjects to bring all study treatment bottles the next visit.

Subjects will be instructed to contact the research facility if they are experiencing any adverse reactions from the medications.

9.2.4 Visit 3

Subjects will report to research site 24 (+/- 2 hrs) hours after initial vorapaxar dose. Remind subjects in groups 2, 3, and 4 to bring all unused study drugs on this visit. The following procedures will be performed:

- Confirm compliance with dietary restrictions (no high fat foods the morning of visit).
- Collection of adverse events.
- Collection of concomitant medications.
- Obtain laboratory specimens for PD measurements for blood through direct venipuncture
- Obtain urine sample for urine thromboxane. After laboratory specimen collection, while in clinic, instruct subject to take vorapaxar 2.08mg, and assigned study treatment (clopidogrel and/or EC ASA). Document in CRF. Provide patient instructions for study treatment. Instruct subject to continue taking study treatment (clopidogrel and/or EC ASA), as assigned, concurrently with vorapaxar 2.08mg for 30 (+/- 7)days. All other home medications will be continued. See specific instructions below as according to subjects' assigned groups.
    - Group 1: vorapaxar 2.08mg orally once a day starting the next day, preferably in the morning.
    - Group 2: vorapaxar 2.08mg and clopidogrel 75mg orally once a day starting the next day, preferably in the morning.
    - Group 3: vorapaxar 2.08mg and EC ASA 81mg orally once a day starting the next day, preferably in the morning.
    - Group 4: vorapaxar 2.08mg, clopidogrel 75mg, and EC ASA orally once a day starting the next day, preferably in the morning.
- Confirm schedule for visit 4.

Subjects will be instructed to contact research facility if they are experiencing any adverse reactions from the medications. Three days prior to visit 4, the study coordinator will call the subjects and reiterate the importance of not missing the study drug/s dose for the next 3 days until the scheduled clinic visit.

9.2.5 Visit 4

Subjects will report to the research site 30 $\pm$ 7 days from initial vorapaxar dose. The day prior to the visit, the study coordinator will call subjects for visit 4 instructions. Remind subjects to avoid any high fat foods, and to hold vorapaxar, EC ASA and/or clopidogrel (for groups 2, 3, and 4) the day of visit 4. For the Endo-PAT2000 test, the subjects need to abstain from smoking and caffeine 2 hours prior to testing. Subjects also need to hold any vasoactive medications 24 hours prior to the test. Remind subject to bring all unused study drugs on this visit and all approved home

medications (to prevent missed concomitant medication doses).The following procedures will be performed during this visit:

- Confirm compliance with dietary restrictions (high fat foods, caffeine).
- Collection of adverse events.
- Collection of unused study medications.
- Review concomitant medications
- Administer Endo-PAT2000 test
- Complete physical exam
- Obtain clinical safety laboratory specimens (comprehensive metabolic panel, complete blood count with platelets and auto differential, hsC-reactive protein)and PD measurements.
- Obtain urine sample for urine analysis and urine thromboxane
- If applicable, after lab work collection and endo-PAT2000test, remind subject to take study drug(s) and approved home medications as according to subject's schedule.
- Study drug accountability
- Dispense study drug for groups 2, 3, and 4 only.
  - Group 2: 17 tablets of clopidogrel 75mg.
  - Group 3: 17 tablets of EC ASA 81mg.
  - Group 4: 17 tablets of clopidogrel 75 mg and EC ASA 81mg.
- Provide patient instructions
  - Stop taking vorapaxar
  - For groups 2, 3, and 4, continue taking EC ASA and/or Clopidogrel (as according to treatment group)
  - Remind subjects not to take any unapproved medications as per protocol.
- Confirm visit 5 schedule

Three days prior to visit 5, the study coordinator will call the subjects and reiterate the importance of not missing the study drug/s dose for the next 3 days until the scheduled clinic visit.

9.2.6 Visit 5

This visit is scheduled 14 + 3 days of visit 4. The day prior to the visit, the study coordinator will call subjects for visit 5 instructions. Remind subjects not to eat any high fat foods, and to hold EC ASA and/or clopidogrel (for groups 2, 3, and 4) the morning of scheduled visit, and to return any unused study drugs. The following procedures will be performed during this visit:

- Confirm compliance with dietary restrictions (high fat foods)
- Collection of adverse events.
- Collection of concomitant medications.
- Blood/urine sample will be obtained for PD measurements.
- Study drug accountability
- Dispense study drug for groups 2, 3, and 4 only.
  - Group 2: 17 tablets of clopidogrel 75mg.

- Group 3: 17 tablets of EC ASA 81mg.
- Group 4: 17 tablets of clopidogrel 75mg and EC ASA 81mg.
- Confirm visit 6 schedule.

Three days prior to visit 6, the study coordinator will call the subject and reiterate the importance of not missing the study drug/s dose for the next 3 days until the scheduled clinic visit.

9.2.7 Visit 6/ End of Study

The subject will report to research site 14 ± 3 days of visit 5. The day prior to the visit, the study coordinator will call the subjects for visit 6 instructions. Remind the subjects not to ingest any high fat foods and to hold EC ASA and/or clopidogrel (for groups 2, 3, and 4) the morning of scheduled visit, and to return any unused study drug. The following procedures will be performed during this visit:

- Vital signs (blood pressure, heart rate) will be obtained after the subject has been in a sitting position for at least 5 minutes.
- Confirm compliance with dietary restrictions (high fat foods)
- Determine adverse event occurrence and follow-up on previously reported adverse events.
- Collection of concomitant medications.
- Blood/urine samples will be obtained for and PD measurements.
- Return unused study drug for groups 2, 3, and 4.
- Study drug accountability
- If applicable, instruct subject to return to original ASA and/or clopidogrel dose.

At the end of the study, all subjects will return to their original standard of care treatment and for any medical treatment questions, will be instructed to resume follow-up with their primary care team.

10 SUBJECT COMPENSATION

The subject or their insurance company will not be billed for this study. All study related tests and procedures will be paid for by the research site. Protocol related drugs will be provided for the duration of the study. A total of $450 financial compensation will be provide for study participation (Visit 1 - $75, Visit 2 - $75, Run-In -$50 ,Visit 3 - $75, Visit 4 - $75, Visit 5 - $75, and Visit 6 - $75) to cover transportation, parking, and meal expenses. Compensation will only be paid for completed visits. Payment will be received by subjects at the time of the visit using reimbursement card or mailed to the subjects via check request.

11 FUNDING

This is an investigator initiated study sponsored by MERCK and Co., Inc. The sponsor will provide investigational product.

12 CONFLICTS OF INTEREST

Paul A Gurbel serves on the medical advisory board for MERCK and Co. and has received $15,000 for the last 12 months, as well as $30,000 speaker fees.

13 FACILITIES AND EQUIPMENT

The research site is equipped with its own laboratory equipment, which includes state of the art technologies for platelet assays and PD measurements, centrifuges, refrigerators, and freezers for investigational specimen processing and storage. Subjects will be seen in the site's outpatient clinic room equipped with an electrocardiogram, Endo-PAT2000 machine, blood pressure equipment, weight scale, and phlebotomy supplies necessary for subject assessment.

14 OUTSIDE CONSULTANTS/COLLABORATORS

There are no outside consultants/collaborators participating.

15 CONTRACTURAL AGREEMENTS

There are no outside consultants/collaborators participating.

16 REFERENCES

1. Magnani G, Bonaca MP, Braunwald E, Dalby AJ, Fox KA, Murphy SA, Nicolau JC, Oude Ophuis T, Scirica BM, Spinar J, Theroux P, Morrow DA. Efficacy and safety of vorapaxar as approved for clinical use in the United States. J Am Heart Assoc. 2015;4:e001505.
2. Gurbel PA, Bliden KP, Guyer K, Cho PW, Zaman KA, Kreutz RP, Bassi AK, Tantry US. Platelet reactivity in patients and recurrent events post-stenting: results of the PREPARE POST-STENTING Study. J Am Coll Cardiol. 2005;46:1820-6.
3. Mehran, R., Rao, S.V., Bhatt, D.L., Gibson, C.M., Caixeta, A., Eikelboom, J., Kaul, S., Wiviott, S.D., Menon, V., Nikolsky, E. Standardized bleeding definitions for cardiovascular clinical trials: a consensus report from the Bleeding Academic Research Consortium. Circulation 2011;123:2736-2747.

17 APPENDICES

17.1 Appendix A: Bleeding Academic Research Consortium (BARC) definition for Bleeding

Type 0 • No 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 health-care professional; may include episodes leading to self-discontinuation of medical therapy by the patient without consulting a health-care professional.
Type 2 • Any overt, actionable sign of hemorrhage (e.g., more bleeding than would be expected for a clinical circumstance, including bleeding found by imaging alone) that does not fit the criteria for type 3, 4, or 5 but does meet at least one of the following criteria: o requiring nonsurgical, medical intervention by a health-care professional, leading to hospitalization or increased level of care, or prompting evaluation
Type 3 • **Type 3a:** o Overt bleeding plus hemoglobin drop of 3 to < 5 g/dL* (provided hemoglobin drop is related to bleed) o Any transfusion with overt bleeding • **Type 3b:** o Overt bleeding plus hemoglobin drop ≥5 g/dL* (provided hemoglobin drop is related to bleed), o Cardiac tamponade, o Bleeding requiring surgical intervention for control (excluding dental/nasal/skin/hemorrhoid), o Bleeding requiring intravenous vasoactive agents • **Type 3c:** o Intracranial hemorrhage (does not include microbleeds or hemorrhagic transformation, does include intraspinal), o Subcategories confirmed by autopsy or imaging or lumbar puncture, o Intraocular bleed compromising vision.
Type 4 • CABG-related bleeding, • Perioperative intracranial bleeding within 48 h, • 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-h period, • Chest tube output more than or equal to 2L within a 24-h period
Type 5 (Fatal bleeding) • **Type 5a:** Probable fatal bleeding; no autopsy or imaging confirmation but clinically suspicious

- **Type 5b:** Definite fatal bleeding; overt bleeding or autopsy or imaging confirmation

17.2 Appendix B: CYP3A4 Inhibitors and Inducers

CYP3A4 Inhibitors	
Amiodarone	Imatinib
Amprenavir	Indinavir
Aprepitant	Isoniazid
Atazanavir	Itraconazole
Chloramphenicol	Ketoconazole
Clarithromycin	Lapatinib
Conivaptan	Miconazole
Cyclosporine	Nefazodone
Darunavir	Nelfinavir
Dasatinib	Posaconazole
Delavirdine	Ritonavir
Diltiazem	Quinupristin
Erythromycin	Saquinavir
Fluconazole	Tamoxifen
Fluoxetine	Telithromycin
Fluvoxamine	Troleandomycin
Fosamprenavir	Verapamil
Grapefruit juice	Voriconazole

CYP3A4 Inducers	
Aminoglutethimide	Nevirapine
Bexarotene	Oxcarbazepine
Bosentan	Phenobarbital
Carbamazepine	Phenytoin
Dexamethasone	Primidone
Efavirenz	Rifabutin
Fosphenytoin	Rifampin
Griseofulvin	Rifapentine
Modafinil	St. John's wort
Nafcillin	

This section should contain all pertinent documents associated with the management of the study. The following list examples of potential attachments:

1. *Investigator Agreement (for any investigator, other than sponsor-investigator, who participates in the study)*
2. *Sample Consent Form*
3. *Study Procedures Flowchart/Table*

4. *Core Lab Instructions To Investigators*
5. *Specimen Preparation And Handling (e.g. for any specialized procedures that study team must follow to process a study specimen, and/or prepare it for shipment)*