Prolocor, Inc.*

Study Protocol, Amendment 3.1

Protocol Number: PRL-0001

Assessment of Individual Risk of Cardiovascular Events by Platelet FcGammaRIIa

NCT05175261

A Prospective Observational Cohort Study

*Prolocor, Inc. is an organization based in the United States of America and is the legal entity acting as the sponsor for this study. The term "sponsor" is used throughout the protocol to represent Prolocor, Inc; the sponsor is identified on the Contact Information page that accompanies the protocol.

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Prepared by: Prolocor, Inc.
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GCP Compliance: This study will be conducted in compliance with Good Clinical Practice, and applicable regulatory requirements.

Confidentiality Statement

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REVISION HISTORY

Ver. No.	Description of Change(s)	Effective Date
2.0	Section 1.3, Schedule of Activities and Section 4.1, Overall Design: The amount of blood obtained for analysis of platelet FcγRIIa changed from 'approximately 30 ml or 2 tablespoons' to 'approximately 3 ml to 6 ml.'	1 December 2021
	Section 1.1 Synopsis, Section 4.1 Overall Design and Section 4.2 End of Study Definition: The duration of subject follow-up was clarified. Text was changed from 'the last subject enrolled has completed 18 months of follow-up' to 'the last subject enrolled has completed at least 18 months of follow-up.'	
	Section 9.1 Statistical Hypotheses: Updated study hypotheses to match those in Section 2.2 Study Hypothesis. Text updated to read as follows: 1. Platelet expression of FcγRIIa is associated with risk of MI, stroke, and death 2. Incorporating platelet expression of FcγRIIa into existing risk scores improves identification of patients at high risk of MI, stroke, and death 3. Platelet expression of FcγRIIa is not associated with risk of major bleeding	
	Miscellaneous minor changes to punctuation, grammar and abbreviations made throughout document.	
3.0	Section 1.1 Synopsis: The age of subjects eligible and type of MI (type 1) were clarified. Text updated to read as follows: Approximately 800 male and female subjects ≥ 18 years of age with confirmed type 1 MI [ST-segment elevation MI (STEMI) or non-ST-segment elevation MI (NSTEMI)] will be enrolled before hospital discharge for the index event	3 February 2022
	Section 5.1 Inclusion Criteria: The age of subjects eligible and type of MI (type 1) were clarified. Text updated to read as follows: 1. Male or female subjects ≥ 18 years of age hospitalized with confirmed type 1 MI (STEMI or NSTEMI), referred to as the index event (described in Appendix 1)	
	Section 1.2 Study Schematic, Section 4.1 Overall Design, and Figure 5 Schematic Overview of the Study: Specified that the type of MI for study eligibility is type 1 MI.	

Ver. No.	Description of Change(s)	Effective Date
4.0	Section 1.1 Synopsis, Section 2.3 Evidence for Use of FcγRIIa as a Tool, and Section 4.1 Overall Design: Updated to allow for limited enrollment of select subjects receiving anticoagulants. Text has been added as follows: Enrollment of subjects who are receiving anticoagulants for atrial fibrillation or venous thromboembolic disease will be limited to approximately 100 subjects.	20 October 2022
	Section 1.1 Synopsis and Section 4.1 Overall Design: Increased the number of participating sites from 'approximately 10' to 'approximately 10-20'.	
	Section 1.3 Schedule of Activities, Section 4.1 Overall Design and Section 8.1 Efficacy and Safety Assessments: Expanded the time window for blood draw for quantification of FcγRIIa. The following text has been added: Blood can be taken up to 2 weeks post-study enrollment.	
	Section 5.2 Exclusion Criteria: Deleted Exclusion Criterion 1 'Requirement for treatment with full dose-anticoagulant therapy (e.g., for atrial fibrillation).	
4.1 (Site Specific)	1.1 Synopsis: Added information on the substudy including objective, endpoint and study population. The following text has been added: Substudy: A substudy will be performed at some sites. Substudy Objective: Demonstrate that the FcγRIIa assay provides a consistent measure of platelet expression of FcγRIIa by measuring a second blood sample after study enrollment. Substudy Endpoint: Measurement of platelet expression of FcγRIIa 2 to 6 weeks after study enrollment which will be compared to FcγRIIa results from the first/baseline blood sample. Study Population: Subjects enrolled in the parent study are eligible	See Header
	1.3 Schedule of Activities: Added blood sample to quantify FcγRIIa 2 to 6 weeks after enrollment into the study.	
	Section 4.3 Substudy. New section added to describe the background, objective, endpoint and subject eligibility of the substudy. The following text has been added: Platelet function tests exhibit substantial intra-individual variability when measures are repeated 2 to 6 weeks after initial testing (Frelinger 2013, Hochholzer 2014, Nührenberg 2015). Results from a study in healthy subjects suggest that FcγRIIa will vary by <10% over the course of 2 to 6 weeks (McMahon 2019). The objective of the substudy is to demonstrate that the FcγRIIa assay provides a consistent measure of platelet expression of FcγRIIa by measuring a second blood sample after study enrollment and the first/baseline blood sample is	

	taken. The substudy endpoint is the measurement of platelet expression of FcγRIIa 2 to 6 weeks after study enrollment. The substudy will be performed at some sites identified by early study start-up and prior experience in similar studies. Subjects enrolled in the parent study are eligible for the substudy. A second sample of blood will be obtained by phlebotomy 2 to 6 weeks after the subject is enrolled in the study. The results from this second sample of blood will be compared to FcγRIIa results from the first/baseline blood sample. See Section 2.4 for the risk/benefit assessment associated with phlebotomy and the second blood draw.	
	Section 9.4.7 Substudy Analyses. New section added regarding substudy analyses. The following text has been added: Details on the substudy statistical analyses will be summarized in the SAP.	

ABBREVIATIONS

ACS	Acute Coronary Syndrome
AE	Adverse Event
ASA	Acetylsalicylic Acid; Aspirin
BARC	Bleeding Academic Research Consortium
ARCTIC	Assessment by a Double Randomization of a Conventional Antiplatelet Strategy versus a Monitoring-guided Strategy for Drug-Eluting Stent Implantation and of Treatment Interruption versus Continuation One Year after Stenting
CFR	Code of Federal Regulations
CKD	Chronic Kidney Disease
CI	Confidence Interval
CRF	Case Report Form
CV	Coefficient of Variation
DAPT	Dual Antiplatelet Therapy
DM	Diabetes Mellitus
Fc	Fragment Constant
eCRF	Electronic Case Report Form
FcγRIIa	FcGammaRIIa
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GRAVITAS	Gauging Responsiveness with A VerifyNow assay-Impact on Thrombosis And Safety
HIPAA	Health Insurance Portability and Accountability Act
HR	Hazard Ratio
ICH	International Conference on Harmonisation
Ig	Immunoglobulin
IRB	Institutional Review Board
MI	Myocardial infarction
ml	milliliter
MVD	Multi-Vessel Coronary Artery Disease
ng	nanogram
nM	nanomolar
NSTEMI	Non-ST-segment elevation myocardial infarction
PCI	Percutaneous Coronary Intervention
QC	Quality Control
REDCap	Research Electronic Data Capture
REDUCE	Evaluation of short-term dual antiplatelet therapy in patients with acute coronary syndrome treated with a new-generation stent
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMART-DATE	6-month versus 12-month or longer dual antiplatelet therapy after percutaneous coronary intervention in patients with acute coronary syndrome
STEMI	ST-segment elevation myocardial infarction
SUSAR	Suspected Unexpected Serious Adverse Reaction
TIMI	Thrombolysis in Myocardial Infarction
TWILIGHT	Ticagrelor with or without Aspirin in High-Risk Patients after PCI
µg	microgram
U	Units
US	United States

STATEMENT OF COMPLIANCE

This study will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or 21 CFR Part 312.)

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from subjects who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

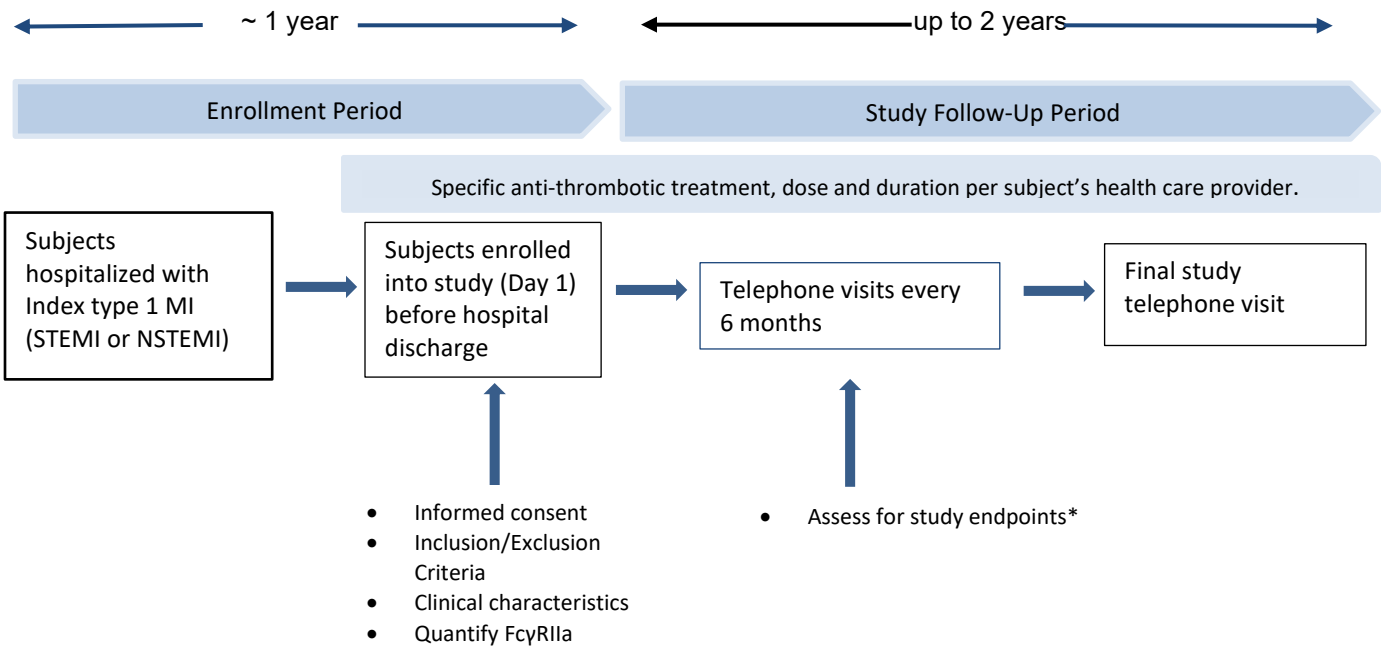
1.1 SYNOPSIS

Title:	Assessment of Individual Risk of Cardiovascular Events by Platelet FcGammaRIIa (FcγRIIa)
Study Description:	A Prospective, Observational Multicenter Non-Interventional Cohort Study
Objectives:	The primary objective is to determine whether platelet expression of FcγRIIa is associated with risk of myocardial infarction (MI), stroke and death. Secondary objectives include: • Develop a score that combines clinical characteristics plus platelet expression of FcγRIIa to determine the risk of MI, stroke, and death. • Determine whether platelet expression of FcγRIIa is associated with risk of major bleeding.
Endpoints:	The primary endpoint is the composite of death, MI, and stroke. A secondary endpoint is the incidence of clinically significant bleeding according to the Bleeding Academic Research Consortium (BARC) scale type 2-5.
Study Population:	Approximately 800 male and female subjects ≥ 18 years of age with confirmed type 1 MI [ST-segment elevation MI (STEMI) or non-ST-segment elevation MI (NSTEMI)] will be enrolled before hospital discharge for the index event. Subjects with increased risk of an event, defined as ≥2 of the following risk factors, will be eligible: • Age ≥65 • Multi-vessel coronary artery disease (MVD) defined as ≥2 vessels or left main with a stenosis ≥50% • Chronic Kidney Disease (CKD) defined as estimated glomerular filtration rate (GFR) <60 ml/min/1.73 m^2 • Diabetes mellitus (DM) • Prior MI Enrollment of subjects who are receiving anticoagulants for atrial fibrillation or venous thromboembolic disease will be limited to approximately 100 subjects.
Phase:	Not Applicable
Number of Sites	Approximately 10-20 sites in the United States will participate in this study.
Study Intervention:	Not Applicable
Study Duration:	It is anticipated that it will take approximately 12 months to enroll approximately 800 subjects. The study and subject follow-up will continue until 1) at least 80 ischemic events (MI, stroke, and death) have occurred, and 2) the last subject enrolled has completed at least 18 months of follow-up.
Participant Duration:	After enrollment, all subjects will be followed until the end of the study that will be at least 18 months after enrollment of the last subject. Based on an annual ischemic event rate of 10%, the overall length of participation in the study is anticipated to be up to 3 years.

Substudy:	A substudy will be performed at some sites.
Substudy Objective:	Demonstrate that the FcγRIIa assay provides a consistent measure of platelet expression of FcγRIIa by measuring a second blood sample after study enrollment.
Substudy Endpoint:	Measurement of platelet expression of FcγRIIa 2 to 6 weeks after study enrollment which will be compared to FcγRIIa results from the first/baseline blood sample
Study Population:	Subjects enrolled in the parent study are eligible.

1.2 STUDY SCHEMATIC

Figure 1 Schematic Overview of the Study



MI=myocardial infarction; NSTEMI= non-ST-segment elevation MI, STEMI= ST-segment elevation MI
* Subjects who experience an endpoint event will continue to be followed throughout the entire study and any subsequent events will also be confirmed and recorded.

1.3 SCHEDULE OF ACTIVITIES

Procedures	Day 1 (prior to or on day of hospital discharge)[a]	Week 2 to Week 6 after study enrollment	Telephone Study Visit every 6 months (+/- 4 weeks)	Final Telephone Study Visit (+ 2 weeks after at least 80 ischemic events accrued)
Informed Consent	X			
Inclusion/Exclusion Criteria	X			
Clinical Characteristics	X			
Enrollment	X			
Anti-thrombotic Medication Review	X		X	X
Blood Sample to Quantify FcγRIIa[b]	X	X[d]		
Assess for Study Endpoints[c]			X	X
Complete Case Report Forms (CRFs)	X		X	X

a: Procedures can be done at any time after confirmation of myocardial infarction index event and prior to hospital discharge. Day 1 is defined as the day the subject is enrolled into the study and must occur prior to or on the day of hospital discharge.
b: Approximately 3 ml to 6 ml blood sample. Blood can be taken up to 2 weeks post-study enrollment.
c: Endpoints include Ischemic events (MI, stroke, death) and clinically significant bleeding BARC type 2-5.
d: For sites participating in the substudy, a 3 ml to 6 ml blood sample will be drawn 2 to 6 weeks after enrollment into the study

2 INTRODUCTION

2.1 BACKGROUND

The American Heart Association estimates that during 2020 approximately 720,000 Americans will have a first coronary event (defined as first hospitalized myocardial infarction (MI) or coronary heart disease death), and approximately 335,000 will have a recurrent event (Benjamin 2019). A retrospective cohort study of 78,085 Medicare beneficiaries ≥ 66 years of age found that 20.6% of patients with MI had at least one re-hospitalization during the 10 years after their index MI (Levitan 2016).

According to Patel and colleagues, a diverse array of therapeutic options is available to clinicians (Patel 2018) and they propose that what is needed now is effective precision medicine strategies to enable clinicians to target patients with high residual risk with more powerful therapy (Patel 2018). The current study is designed to use translational research to develop biomarkers that may effectively guide individualized therapy based on the hypothesis that platelet expression of FcγRIIa will identify patients at high and low risk of subsequent MI, stroke, and death. Validation of platelet expression of FcγRIIa as a biomarker of residual cardiovascular risk will support subsequent studies designed to use platelet expression of FcγRIIa as a precision medicine tool.

Strategies to Reduce Recurrent Ischemic Events/Bleeding

Strategies designed to reduce the risk of recurrent ischemic events and bleeding are predicated on the following assumptions: 1) The benefit-risk of a treatment should be assessed by comparing the absolute risk differences of the benefit and harm endpoints. 2) A treatment's benefit is generally consistent across population on a relative scale. For this reason, on an absolute scale, benefit is generally greater in high-risk populations. 3) A treatment's harm is generally consistent across populations on an absolute scale. For this reason, benefit-risk is generally greater in a high-risk population. 4) Accordingly, a marker that identifies a high-risk population can enable a treatment to be used in a way that increases its benefit-risk profile. One strategy designed to reduce the risk of recurrent ischemic events is the continuation of dual antiplatelet therapy (DAPT). Two large multicenter randomized trials have demonstrated that long-term DAPT reduces the risk of subsequent cardiovascular events. Treatment with aspirin (ASA) plus clopidogrel reduced the risk of major adverse cardiovascular and cerebrovascular events from 5.9% with ASA monotherapy to 4.3% ($p<0.001$) with ASA plus clopidogrel (Mauri 2014). Continued DAPT increased the risk of moderate or severe bleeding (2.5% vs. 1.6%, $p=0.001$). Treatment with ASA plus ticagrelor (60 mg twice daily) reduced the risk of cardiovascular events to 7.8% compared with 9.0% with ASA alone (Bonaca 2015). The risk of Thrombolysis in Myocardial Infarction (TIMI) major bleeding was higher with ticagrelor (2.3% with 60 mg twice daily) compared with ASA alone (1.1%). Accordingly, the benefits of long-term treatment with DAPT are at least partially offset by an increase in bleeding. Because of this trade-off, the use of DAPT long-term is limited. The promise of precision medicine is to reduce the overall incidence of bleeding by individualizing therapy, targeting patients with high residual cardiovascular risk for long-term DAPT.

The “Ticagrelor with or without Aspirin in High-Risk Patients after PCI (TWILIGHT)” study tested a strategy of ticagrelor monotherapy compared with aspirin plus ticagrelor and demonstrated a reduced risk of bleeding events without an increase in ischemic events with ticagrelor monotherapy (Mehran 2019). While this strategy reduced bleeding, it did not provide guidance on which patients will derive the greatest benefit from long term treatment with more powerful antiplatelet therapy. Further, because treatment with more powerful anti-thrombotic therapies has been consistently associated with a greater incidence of bleeding, it is likely that monotherapy with ticagrelor will be associated with greater bleeding than ASA monotherapy.

A strategy designed to limit the risk of bleeding events is the early transition from DAPT to ASA monotherapy. The 6-month versus 12-month or longer dual antiplatelet therapy after percutaneous coronary intervention (PCI) in patients with acute coronary syndrome (SMART-DATE) randomized patients with acute coronary syndrome (ACS) to either 6 months of DAPT or at least 12 months of DAPT (Hahn 2018). MI (1.8% vs 0.8%; p=0.02) and stent thrombosis (1.1% vs 0.7%, p=0.09) were more common in the 6-month DAPT group. Bleeding Academic Research Consortium (BARC) type 2–5 bleeding occurred in 2.7% of the 6-month DAPT group and 3.9% of the 12-month or longer DAPT group (p=0.09). In the “Evaluation of short-term dual antiplatelet therapy in patients with acute coronary syndrome treated with a new-generation stent (REDUCE)” trial a composite (ischemia and bleeding) endpoint was non-inferior in 3 months compared with 12 months of DAPT among patients with ACS (De Luca 2019). Once again, a tradeoff was apparent between the risk of bleeding and the risk of thrombosis with numerically fewer bleeding events (3.3% vs 4.0%, p=0.46) associated with a numerically higher incidence of cardiac mortality (1.8% vs 1.1%, p=0.28) and stent thrombosis (1.6% vs 0.8%, p=0.16) with shortened DAPT.

The critical gap facing clinicians is demonstrated by the combination of 1) trials demonstrating that prolonged treatment with DAPT can reduce the incidence of ischemic/thrombotic events at the cost of bleeding; and 2) trials demonstrating that shortened DAPT can reduce the incidence of bleeding at the cost of a greater risk of ischemic events. Consensus guidelines recommend treatment with DAPT after MI and coronary stenting, however, specific guidance on the duration of DAPT is not possible based on available evidence (Bittl 2016). The guideline committee recommended a minimum duration with DAPT of 3 to 6 months but also noted that extension of DAPT beyond 12 months entailed a tradeoff between a risk of bleeding and ischemic events. A precision tool that can be used to guide clinical decision making would improve care. Patients at low risk should benefit from shortened treatment with more powerful antiplatelet therapy whereas patients at high risk should derive greater absolute benefit from long term treatment with more powerful antiplatelet therapy.

Current Clinical Risk Scores

Clinical risk scores do not bridge the critical gap to enable clinicians to individualize long-term treatment with DAPT. For example, Yeh and colleagues developed a predictive score, the DAPT score, which is a tool designed to simultaneously predict ischemic and bleeding risk (Yeh 2016). Registry data from Sweden (2006 to 2014) that followed 41,101 patients demonstrated that the score had a discrimination of 0.54 for cardiovascular and cerebrovascular events, and

0.49 for fatal or major bleeding (Ueda 2018). Thus, the DAPT score supports individualization of therapy, however, the predictive accuracy is not sufficient to effectively guide therapy.

Schneider et.al. applied the DAPT score to patients enrolled in a single center pilot study (Schneider 2020). Patients with DAPT score ≥ 2 had a predicted event rate of 7.6% and an observed event (MI, stroke, death) rate of 10.1%. High platelet FcγRIIa expression was evident in 55% of patients with DAPT ≥ 2. The combination of DAPT ≥ 2 plus high platelet FcγRIIa expression was associated with an event rate of 15.4% whereas the combination of DAPT ≥ 2 plus low platelet FcγRIIa expression was associated with an event rate of 3.7% ($p<0.05$). The broad distribution of platelet FcγRIIa expression in patients with both high and low clinical risk scores and these study results suggest that platelet FcγRIIa expression can be used to discriminate higher and lower risk among patients categorized as high risk by clinical risk tools.

Platelet Function Testing

Because increased platelet reactivity has consistently identified patients at greater risk of subsequent cardiovascular events (Breet 2010, Wisman 2014, Reny 2016), large clinical trials were designed to determine whether platelet function testing could effectively guide antiplatelet therapy. Two trials failed to demonstrate that currently available tests of platelet function can be used to guide treatment (Price 2011, Collet 2012). Specifically, the GRAVITAS trial (Gauging Responsiveness with A VerifyNow assay-Impact on Thrombosis And Safety) randomized patients with high platelet reactivity to standard compared with high dose clopidogrel (Price 2011). One proposed reason for the lack of efficacy in this study was that effective suppression of high platelet reactivity with high dose clopidogrel was not confirmed. The ARCTIC trial (Assessment by a Double Randomization of a Conventional Antiplatelet Strategy versus a Monitoring-guided Strategy for Drug-Eluting Stent Implantation and of Treatment Interruption versus Continuation One Year after Stenting) addressed this concern by employing a treatment strategy in which platelet function testing was used to confirm suppression of high platelet reactivity by augmenting treatment followed by confirmation that high platelet reactivity was suppressed (Collet 2012). The failure of ARCTIC suggests inadequate suppression of high platelet reactivity was not the mechanism responsible for failure. Thus, currently available platelet function tests are not capable of guiding individualized care. Intra-individual variability in platelet function over time is substantial and may be a major contributor to the failure (Frelinger 2013, Hochholzer 2014, Nührenberg 2015) as evidenced in the ELEVATE-TIMI 56 study where more than 40% of patients exhibited a greater than 20% change in platelet function over the course of 2 weeks (Hochholzer 2014).

Summary

Cardiovascular disease is prevalent and recurrent cardiovascular events are a major cause of morbidity and mortality. An expanding armamentarium of therapeutic options that reduce cardiovascular risk underscores the promise of precision medicine that effectively tailors therapy to subsequent risk. Clinical risk tools and currently available tests of platelet function are not sufficient to effectively guide therapy.

2.2 STUDY HYPOTHESIS

Platelet expression of FcγRIIa is hypothesized to be a novel biomarker that, when quantified, can be used to identify patients at high and low risk of cardiovascular events. This hypothesis is based on the premise that currently available platelet function tests are analogous to a random glucose measurement and that platelet expression of FcγRIIa is analogous to an HbA1C, reflecting platelet reactivity over a longer interval of time. Because FcγRIIa amplifies platelet activation (Boylan 2008, Lova 2002) increased platelet expression of FcγRIIa will identify patients with consistently high platelet reactivity (Serrano 2007). Accordingly, high platelet expression of FcγRIIa leverages the clinical risk of increased platelet reactivity demonstrated by more than 100 studies and over 22,000 patients (Breet 2010, Wisman 2014, Reny 2016). Moreover, quantification of platelet FcγRIIa expression eliminates factors that cause variability with platelet function tests including the preparation of blood and the *in vitro* activation of platelets. The measurement of platelet expression of FcγRIIa does not require activation of platelets and platelet FcγRIIa does not exhibit the magnitude of intra-individual variability seen with platelet function tests.

Specifically, the study hypotheses are:

1. Platelet expression of FcγRIIa is associated with risk of MI, stroke, and death.
2. Incorporating platelet expression of FcγRIIa into existing risk scores improves identification of patients at high risk of MI, stroke, and death.
3. Platelet expression of FcγRIIa is not associated with risk of major bleeding.

2.3 EVIDENCE FOR USE OF FCγRIIA AS A TOOL

FcγRIIa, a member of the Fc family, is expressed on the surface of platelets and amplifies platelet activation (Boylan 2008, Lova 2002). Because FcγRIIa amplifies platelet activation (Boylan 2008, Lova 2002), greater expression increases platelet reactivity (Serrano 2007). In a single center pilot study, Schneider et.al. found that high platelet expression of FcγRIIa (≥ 11,000/platelet) was associated with a greater risk (odds ratio > 4) of MI, stroke, and death (Schneider 2018). Platelet expression of FcγRIIa does not require activation of platelets, is not affected by antiplatelet and anticoagulant agents, and does not exhibit the magnitude of intra-individual variability seen with platelet function tests (McMahon 2019). Further, clinical risk scores have demonstrated overlap between the risk of thrombotic/ischemic events and the risk of bleeding events (Hsieh 2017). Increased platelet expression of FcγRIIa was not associated with a greater risk of bleeding (Schneider 2018) and may distinguish thrombotic risk from bleeding risk.

Evidence in support of platelet expression of FcγRIIa as an effective precision tool:

1. Platelet function tests demonstrate substantial intra-individual variability (Frelinger 2013, Hochholzer 2014, Nührenberg 2015), with more than 40% of patients exhibiting a > 20% change in platelet function over the course of 2 weeks (Hochholzer 2014). Platelet FcγRIIa expression exhibits substantially less intra-individual variability (McMahon 2019). Platelet expression of FcγRIIa is determined by megakaryocyte production that was demonstrated to

be increased by interferon γ (Schneider 2009). Thus, expression of FcγRIIa should be consistent throughout the life of the platelet. Less intra-individual variability would be expected and has been observed (Figure 1). Consistent with these results, Calverley and colleagues found an average change in platelet FcγRIIa expression of less than 10% in 248 patients with cardiovascular disease when repeat determinations were performed 6-52 weeks later (Calverley 2002).

2. Greater platelet expression of FcγRIIa is associated with increased platelet reactivity because FcγRIIa amplifies platelet activation (Boylan 2008, Lova 2002). Unlike currently available tests of platelet function that measure platelet reactivity in response to a select agonist or group of agonists, platelet expression of FcγRIIa predicts increased platelet reactivity in response to a variety of agonists (Figure 2).
3. Because determination of platelet FcγRIIa expression entails quantification of a surface marker, it is less sensitive to perturbations related to assay conditions (Table 1) (McMahon 2019). Neither anticoagulants nor a $P2Y_{12}$ antagonist alter platelet expression of FcγRIIa (McMahon 2019, Table 1).

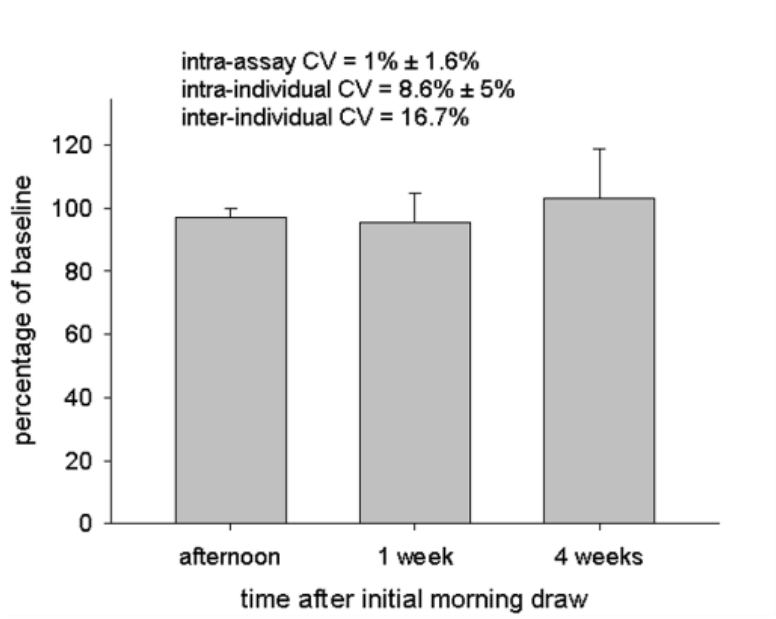


Figure 1: Intra-individual variability in platelet expression of FcγRIIa over the course of 1 month in healthy subjects (n=10). Because platelet function is known to exhibit diurnal variation, platelet expression of FcγRIIa was assessed in the morning and afternoon as well as after 1 week and 1 month (McMahon 2019).

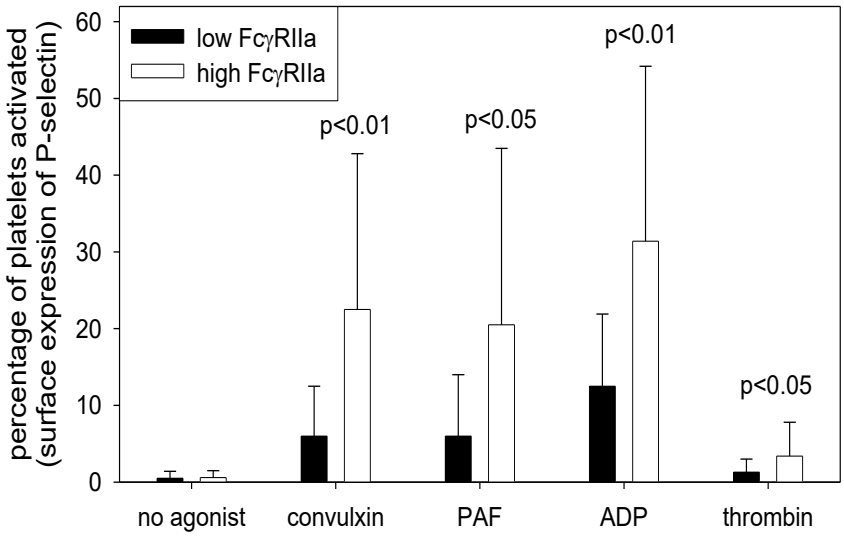


Figure 2: Activation of platelets identified by surface expression of P-selectin with the use of flow cytometry. Patients with end stage renal disease (n=33) were stratified into 2 groups on the basis of median expression of FcγRIIa. The percentage of platelets expressing P-selectin on their surface in the absence of agonist and in response to the collagen mimetic convulxin (1 ng/ml), platelet activating factor (PAF, 1 nM), adenosine diphosphate (ADP 0.2 μM), or thrombin (1 nM) is shown. High platelet expression of FcγRIIa was associated with greater activation of platelets in response to each agonist (Serrano 2007).

Table 1: Intra-assay coefficient of variation (CV) for assay of platelet FcγRIIa expression

CV same anticoagulant	1.9% ± 0.5%
CV between anticoagulants	1.7% ± 0.5%
CV citrate anticoagulant vs citrate + cangrelor	0.7% ± 0.2%

Blood was taken from 3 subjects and anticoagulated with corn trypsin inhibitor (32 μg/ml), trisodium citrate (3.2%), unfractionated heparin (1.2 U/ml), and bivalirudin (8 μg/ml). Cangrelor (500 nM) as added to blood anticoagulated with citrate. Duplicate determination of platelet FcγRIIa expression in each condition (McMahon 2019).

In summary, platelet expression of FcγRIIa is a novel biomarker that appears to identify patients at high and low risk of subsequent cardiovascular events (Schneider 2018). Because FcγRIIa amplifies the activation of platelets (Boylan 2008, Lova 2002) it is a marker of consistently increased platelet reactivity. Thus, platelet expression of FcγRIIa leverages results from multiple studies demonstrating that greater platelet reactivity is associated with a greater risk of cardiovascular events (Breet 2010, Wisman 2014, Reny 2016) with an assay not affected by antiplatelet or anticoagulant therapy that reproducibly identifies consistently increased platelet reactivity and may effectively discriminate residual cardiovascular risk and guide individualized treatment.

FcγRIIa as a novel biomarker

FcγRIIa was identified as the low-affinity receptor for the fragment constant (Fc) portion of immunoglobulin (Ig) G (Karas 1982). Binding to FcγRIIa activates platelets. For example, platelets can coat an Ig-bound (opsonized) entity such as a bacterium via FcγRIIa and this

binding triggers platelet activation and release of secondary mediators that amplify the platelet response to a wide range of bacteria (Cox 2011, Arman 2014). In addition, platelet FcγRIIa is involved in heparin-induced thrombocytopenia and thrombosis, and the principal cellular target for anti-platelet factor 4/heparin antibodies is the platelet FcγRIIa receptor (Kelton 1988, Reilly 2001). FcγRIIa and glycoprotein VI are linked on human platelets, and ligands acting at either receptor activate dual proteolytic regulatory pathways (Gardiner 2008). FcγRIIa markedly enhances thrombus formation when platelets are perfused over a collagen-coated flow chamber under conditions of arterial and venous shear (Zhi 2013). More recently, an additional important function of platelet FcγRIIa has been defined. Phosphorylation of FcγRIIa amplifies the activation of platelets (Boylan 2008, Lova 2002). These observations led to assessing platelet reactivity in patients with end stage renal disease because these patients were known to exhibit increased platelet expression of FcγRIIa (El-Shahawy 2007). The reactivity of platelets with higher and lower platelet expression of FcγRIIa was compared and found that platelets with more FcγRIIa exhibited greater activation in response to sub-maximal concentrations of multiple agonists (Figure 2, Serrano 2007). Taken together, these results led to the hypothesis that high platelet expression of FcγRIIa is a novel biomarker of increased platelet reactivity.

Platelets from healthy young people carry 1,000-4,000 molecules of FcγRIIa (Karas 1982). Calverley and colleagues found that platelet expression of FcγRIIa was increased in patients with an acute coronary or cerebrovascular events as well as in patients with diabetes and individuals without cardiovascular events who had with two or more cardiac risk factors (Calverley 2002). In addition, they found limited intra-individual variability when repeat determination was performed after 6-52 (average 20) weeks (Calverley 2002). These results suggest that, unlike measures of platelet function that exhibit marked intra-individual variability (Frelinger 2013, Hochholzer 2014, Nührenberg 2015), platelet expression of FcγRIIa is a consistent marker of increased platelet reactivity.

Flow cytometry is a powerful tool capable of assessing expression of FcγRIIa on the platelet surface. Platelets are identified by their size as well as a surface marker. Without a standardized measure of fluorescence intensity, results of analyses can be described only in relative terms of fluorescence intensity. The interpretation of fluorescence intensity measurements can be complicated by multiple factors including daily instrument variation, differences in hardware (laser power, filter sets), as well as the density of fluorochrome labeling of antibodies. A goal is to develop a clinically validated tool that can be used broadly in the care of patients, therefore external standards will be employed in the current study to enable the standardization of fluorescence intensity units (Kay 2006, Quadrini 2016). Accordingly, platelet expression of FcγRIIa will be quantified with the use of external standards to quantify the number of molecules on the platelet surface rather than relative platelet expression of FcγRIIa.

Prior study to evaluate FcγRIIa

Schneider et.al. completed a single center prospective trial designed to determine whether platelet expression of FcγRIIa would discriminate cardiovascular risk (Schneider 2018). Patients (n=197) were enrolled shortly before discharge from a hospitalization for MI (both ST elevation and non-ST elevation were included). Although inclusion criteria allowed patients who

had been treated with PCI, coronary artery bypass grafting, or medically, all patients enrolled were treated with PCI. The primary endpoint, a composite of MI, stroke, death, and coronary revascularization, was lower in patients with platelet expression of FcγRIIa <11,000 (Figure 3). All patients were treated with ASA (81 mg) and treatment with clopidogrel (~64%) and ticagrelor (~36%) was balanced in patients with high and low platelet expression of FcγRIIa (Serrano 2007).

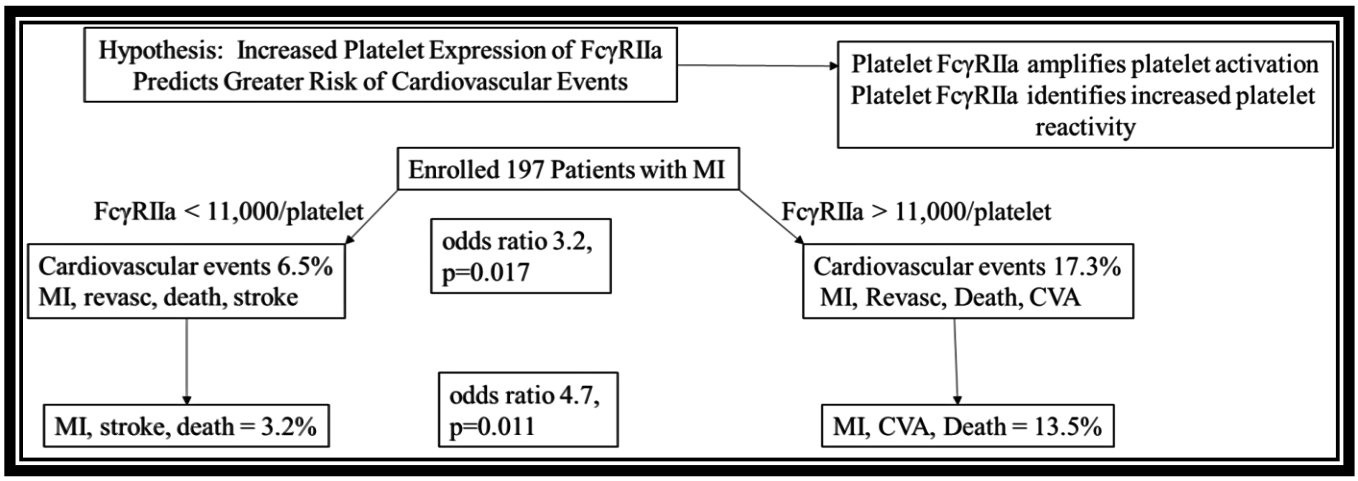


Figure 3: Summary of prospective single center study (Schneider 2018)
CVA=cerebrovascular accident, MI=myocardial infarction, Revasc=revascularization

Clinical characteristics of patients with high and low platelet expression of FcγRIIa were well balanced with the exception of older age, diabetes, and prior revascularization being more prominent in the high expression group. Cox multivariate analysis for the combination of MI, stroke, revascularization, and death demonstrated a hazard ratio of 3.0 (p=0.02) for platelet expression of FcγRIIa >11,000 when age, diabetes, and prior revascularization were included as covariates.

The incidence of revascularization was similar in patients with high and low platelet expression of FcγRIIa (low platelet FcγRIIa n=3, 3.2%; high platelet FcγRIIa n=4, 3.8%, p=0.815). Patients with platelet expression of FcγRIIa ≥11,000 had a greater risk of MI, stroke, and death that became apparent after 6 months (Figure 4).

Cox regression analysis was performed and platelet expression of FcγRIIa was the sole covariate (hazard ratio 3.9, p=0.035) associated with freedom from MI, stroke, and death.

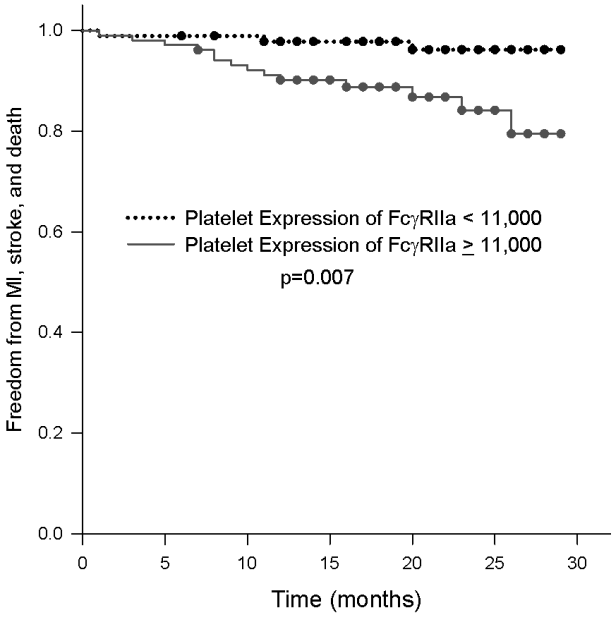


Figure 4: Kaplan-Meier curves of the probability of freedom from MI, stroke, and death. The average duration of follow-up was 20 months (range 6-29 months).
MI=myocardial infarction

The predictive capacity of platelet expression of FcγRIIa was evaluated. The sensitivity of high expression to identify patients with cardiovascular events was 0.82 (95% CI 0.57 - 0.92) and the specificity was 0.51 (95% CI 0.43 - 0.58). MI, stroke, and death were uncommon (8% of all patients) in patients who had had an MI. The negative predictive value of low platelet expression of FcγRIIa was 0.97 (95% CI 0.89 - 0.98) and the positive predictive value of high platelet expression of FcγRIIa was 0.14 (95% CI 0.10 - 0.46). While identification of patients at high risk has great value, the identification of patients at low risk is important to reassure clinicians that they can safely withhold therapy that is associated with a risk of complications. As is often the case for low prevalence events, the negative predictive value of platelet FcγRIIa expression (97% [95% CI 89% - 98%]) offers potentially important guidance to clinicians.

A key objective of this study is to determine the optimal threshold of platelet FcγRIIa expression that classifies patients as high and low risk that will, in turn, be incorporated into an individualized score designed to predict patient risk. Assessment of platelet FcγRIIa expression as a continuous variable is expected to be more predictive than as a dichotomous variable. If assessment of platelet FcγRIIa expression as a dichotomous variable is nearly as predictive as a continuous variable, then the simplicity associated with the definition of a threshold for high and low risk could be preferred.

Clinical criteria that identify patients at increased risk of thrombosis also identify an increased risk of bleeding (Lindholm 2019). A key feature predisposing to both thrombosis and bleeding is older age. Not surprisingly, patients identified as having an increased risk of ischemic events also exhibit a higher risk of bleeding events (Hsieh 2017, Lindholm 2019). In this pilot study, increased platelet expression of FcγRIIa was not associated with a greater risk of bleeding

(Schneider 2018). Further, a meta-analysis of platelet function tests demonstrated that patients with high platelet reactivity were at lower risk of bleeding events compared with those who exhibit low platelet reactivity (Aradi 2015).

In summary, preliminary results demonstrate:

- Increased platelet FcγRIIa expression is associated with consistently increased platelet reactivity
- With appropriate calibration platelet expression of FcγRIIa can be quantified to enable comparison between centers and to assess changes over time
- Unlike platelet function tests, measurement of platelet expression of FcγRIIa is not substantially affected by conditions of the assay, antiplatelet therapy nor anticoagulant therapy
- Platelet expression of FcγRIIa ≥ 11,000/platelet identifies patients with ~4-fold greater risk of MI, stroke, and death

Current Study

Confirmation of results seen in the single center pilot study is necessary in a larger multicenter trial to validate the results of the pilot study and to determine whether platelet expression of FcγRIIa can be used to guide individualized precision therapy and improve outcomes. Results from the current study will determine the prognostic implications of platelet FcγRIIa expression as well as the optimal manner in which to use platelet FcγRIIa expression. The analysis plan will include assessment of platelet FcγRIIa expression as a continuous variable as well as a sensitivity analysis designed to determine whether FcγRIIa ≥ 11,000/platelet is the optimal threshold for discrimination of risk. In addition, it will it provide key preliminary evidence regarding prognostic implications of platelet FcγRIIa expression associated with anti-thrombotic regimens and support plans for a subsequent clinical trial designed to use platelet FcγRIIa expression as a precision tool.

To identify subjects at greater risk of events, subjects will be enrolled who have an increased risk of cardiovascular events based on results from the SWEDEHEART registry that analyzed 100,879 patients (Lindholm 2019). Hospitalized MI subjects with ≥ 2 of the following risk factors; age ≥ 65, prior MI, diabetes mellitus, multivessel coronary artery disease (two or more vessels or left main with a stenosis ≥50% at coronary angiography) and chronic kidney disease (an estimated glomerular filtration rate below 60 ml/min/1.73 m^2) will be enrolled in the current study. In the SWEDEHEART registry, patients with ≥ 2 risk factors comprised 47% of patients and their risk of death, MI and stroke was 9.7/100 patient years. In the pilot study, 55% of subjects with increased risk based on clinical risk scores exhibited platelet expression of FcγRIIa ≥11,000 (Schneider 2020). In the current study. enrollment of subjects receiving treatment with anticoagulant therapy (i.e., for treatment of atrial fibrillation, venous thromboembolism) will be limited to approximately 100 subjects. Subjects with non-cardiovascular conditions expected to limit survival to less than 2 years will be excluded in the current study.

Because high platelet expression of FcγRIIa increases platelet reactivity (Boylan 2008, Lova 2002), it is anticipated that despite an association between high platelet expression of FcγRIIa

and older age (Schneider 2018), there will not be a significantly increased incidence of clinically significant bleeding, defined as BARC type 2-5, among patients with high platelet expression of FcγRIIa.

Lastly, to ensure consistent results, platelet expression of FcγRIIa will be quantified at a core laboratory. At clinical sites, citrate anticoagulated blood will be added to fixative tubes. These tubes will be shipped to the core laboratory for quantification of platelet FcγRIIa expression.

2.4 RISK/BENEFIT ASSESSMENT

2.4.1 KNOWN POTENTIAL RISKS

There is a limited potential for risks associated with phlebotomy, a commonly performed procedure that will be performed exclusively by individuals specially trained in this procedure. Standard phlebotomy techniques to minimize venipuncture side effects (hand hygiene, glove use, skin antisepsis, and the use of sterile, single-use needles will minimize the risk of infection; appropriate angle of needle entry to avoid hematoma, application of pressure for 3-5 minutes after drawing blood). There is a risk of discomfort, bleeding which is generally mild, bruising, and a remote risk of infection that will be minimized by using these techniques

2.4.2 KNOWN POTENTIAL BENEFITS

Subjects enrolled in this study will not derive direct benefit. Data from this study may help inform future studies or provide information for individualized therapy.

3 OBJECTIVES AND ENDPOINTS

The primary objective of the study is to determine whether platelet expression of FcγRIIa is associated with risk of MI, stroke, and death.

Secondary objectives are

- Develop a score that combines clinical characteristics plus platelet expression of FcγRIIa to determine the risk of MI, stroke, and death.
- Determine whether platelet expression of FcγRIIa is associated with risk of major bleeding.

The primary endpoint is the composite of death, MI, and stroke. A secondary endpoint is clinically significant bleeding according to BARC type 2-5.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is a prospective, observational multicenter non-interventional cohort study designed to integrate into the regular standard of care (Figure 5, Schematic Overview). Subjects who are admitted to the hospital with an MI will be screened. Screening will entail review of the medical

record for selected entry criteria. If the subject meets entry criteria, written informed consent will be obtained. Subjects will be enrolled into the study (called Day 1) before discharge from a hospitalization for a confirmed type 1 MI (STEMI or non-STEMI), referred to as the index event. Subjects will not have their care altered by participation in the study; subjects' medical regimens will be determined by their health care provider. Enrollment of subjects who are receiving anticoagulants for atrial fibrillation or venous thromboembolic disease will be limited to approximately 100 subjects.

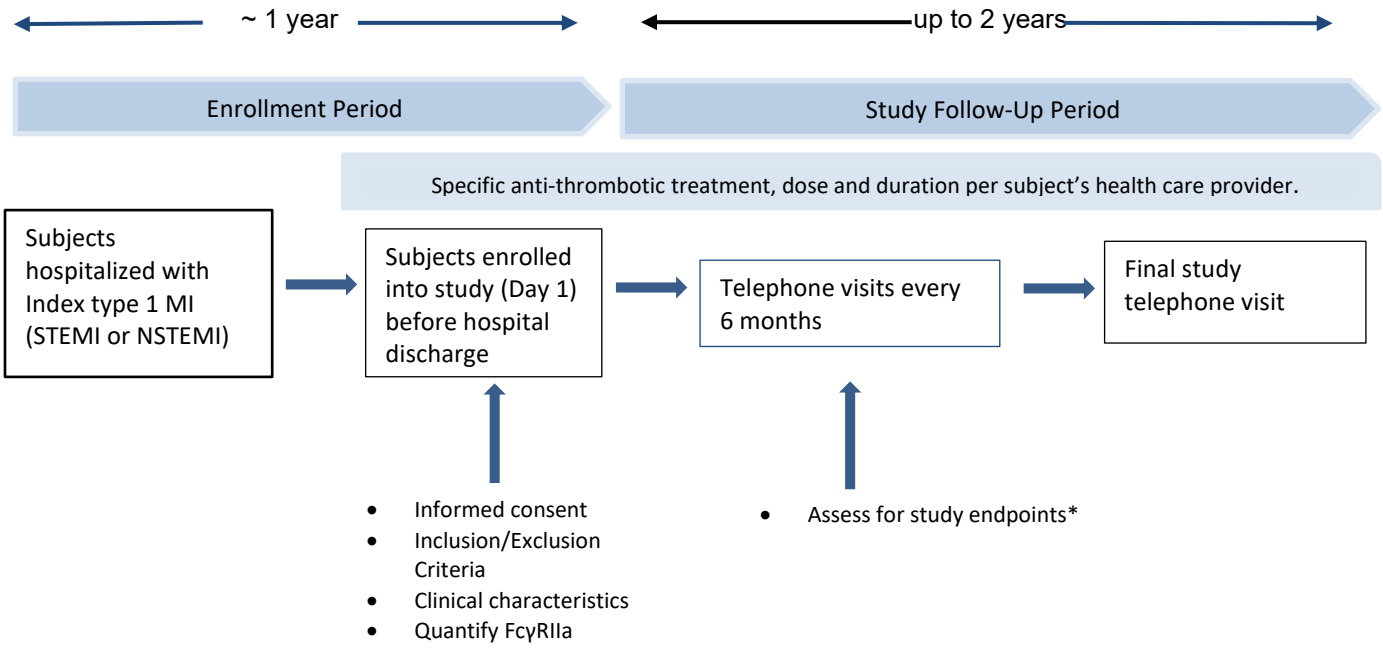
After informed consent is obtained and the subject eligibility is confirmed, each subject will be assigned a unique identifier. Clinical characteristics and biochemical results will be associated with the unique identifier. The association between the subject's identity and the unique identifier will be kept in a secure file. Clinical characteristics will be recorded and blood (approximately 3 ml to 6 ml) will be obtained for analysis of platelet FcγRIIa expression. Blood can be taken up to 2 weeks post-study enrollment. The samples for platelet FcγRIIa expression will be assayed by a core laboratory to ensure standardization.

Subjects will be contacted by telephone every 6 months and asked a standardized set of questions to determine whether they have had a cardiovascular event (MI, stroke), bleeding event or have died. At each study visit, subjects will be queried about their medications with a specific focus on their anti-thrombotic regimen. Changes to their anti-thrombotic regimen will be documented in association with the time when changes were made. All subjects will be contacted at the end of the study for a final telephone visit. Medical records will be reviewed to confirm subject reported endpoints.

The primary endpoint will be the composite of death, MI, and stroke. MI will be defined in accordance with the 4th universal definition (Thygesen 2018) and stroke consistent with consensus guidelines (Sacco 2013). The incidence of clinically significant bleeding will be determined using the Bleeding Academic Research Consortium (BARC) scale (Mehran 2011) and is defined as BARC type 2-5. Study endpoint events will be confirmed by the review of medical records by the investigator and all relevant medical records will be obtained by the site for submission to the sponsor (as described in Section 10.8). Subjects who experience an endpoint event will continue to be followed throughout the entire study and any subsequent endpoint events will also be confirmed and recorded. See Appendix 1 for study endpoint event definitions.

A total of approximately 800 subjects will be enrolled at approximately 10-20 sites in the US. It is anticipated that it will take about 12 months to enroll the total number of subjects. The study and subject follow-up will continue until 1) at least 80 ischemic events (MI, stroke, and death) have occurred, and 2) the last subject enrolled has completed at least 18 months of follow-up. Based on an annual ischemic event rate of 10%, the overall length of participation in the study is anticipated to be up to 3 years. Results will be monitored during the course of the study.

Figure 5 Schematic Overview of the Study



MI=myocardial infarction; NSTEMI= non-ST-segment elevation MI, STEMI= ST-segment elevation MI
* Subjects who experience an endpoint event will continue to be followed throughout the entire study and any subsequent events will also be confirmed and recorded.

4.2 END OF STUDY DEFINITION

The study will be stopped when 1) at least 80 ischemic events (MI, stroke, and death) have been confirmed and 2) the last subject enrolled has completed at least 18 months of follow-up. The study will be considered complete once all subjects have had their last telephone visit.

4.3 SUBSTUDY

Platelet function tests exhibit substantial intra-individual variability when measures are repeated 2 to 6 weeks after initial testing (Frelinger 2013, Hochholzer 2014, Nührenberg 2015). Results from a study in healthy subjects suggest that FcγRIIa will vary by <10% over the course of 2 to 6 weeks (McMahon 2019).

The objective of the substudy is to demonstrate that the FcγRIIa assay provides a consistent measure of platelet expression of FcγRIIa by measuring a second blood sample after study enrollment and the first/baseline blood sample is taken. The substudy endpoint is the measurement of platelet expression of FcγRIIa 2 to 6 weeks after study enrollment.

The substudy will be performed at some sites identified by early study start-up and prior experience in similar studies. Subjects enrolled in the parent study are eligible for the substudy. A second sample of blood will be obtained by phlebotomy 2 to 6 weeks after the subject is enrolled in the study. The results from this second sample of blood will be compared to FcγRIIa results from the first/baseline blood sample.

See Section 2.4 for the risk/benefit assessment associated with phlebotomy and the second blood draw.

5 STUDY POPULATION

Subjects meeting all the inclusion criteria and none of the exclusion criteria are eligible for enrollment into the study.

5.1 INCLUSION CRITERIA

1. Male or female subjects ≥ 18 years of age hospitalized with confirmed type 1 MI (STEMI or NSTEMI), referred to as the index event (described in Appendix 1).
2. Must have ≥ 2 of the following risk factors:
    a) Age ≥ 65
    b) Multi-vessel coronary artery disease (MVD) defined as ≥2 vessels or left main with a stenosis ≥50%
    c) Chronic kidney disease (CKD) defined as estimated glomerular filtration rate (GFR) < 60 ml/min/1.73 m^2
    d) Diabetes mellitus (DM)
    e) Prior MI
3. Must agree to participate in the study, to comply with all study procedures and follow-up contact
4. Signed the informed consent form

5.2 EXCLUSION CRITERIA

1. Participation in another trial in which the subject is known to receive or could receive anticoagulant or antiplatelet treatment as part of the trial intervention.
2. Non-cardiovascular conditions that, in the judgment of the investigator, will limit survival to less than 2 years

5.3 SCREEN FAILURES

Subjects who do not meet the criteria for participation in this study (screen failure) may not be rescreened during the same hospital admission period. The reason for the screen failure should be recorded in the site study log.

5.4 RECRUITMENT

Efforts will be made to enroll an ethnically diverse cohort with appropriate representation from women.

6 STUDY INTERVENTION

There is no study intervention. Treatment decisions are entirely at the discretion of the subject's health care provider.

6.1 MEDICATIONS

Anti-thrombotic therapy will be recorded in the case report form (CRF).

7 SUBJECT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Subjects are free to withdraw from participation in the study at any time upon request. The reason for subject discontinuation or withdrawal from the study will be recorded on the CRF.

7.1 LOST TO FOLLOW-UP

Every effort will be made to minimize the number of subjects who are lost to follow-up. The following actions must be taken if a subject misses a required study visit:

- The site will attempt to contact the subject and reschedule the missed visit and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain if the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the subject (3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record or study file.
- Should the subject continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.
- Electronic medical records may be used to assess vital status (alive or dead) at the end of the study provided the subject has not withdrawn consent for follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY AND SAFETY ASSESSMENTS

This is an observational non-interventional study, therefore there is no evaluation of efficacy or safety. Blood will be taken prior to discharge from the hospital for the index MI event (see separate Laboratory Sample Manual for details on sample collection, storage, and shipment to a core laboratory) and will used to quantify FcγRIIa. Blood can be taken up to 2 weeks post-study enrollment. Risks associated with phlebotomy include discomfort, bleeding, and a remote risk of infection.

Subjects will receive medications as determined by their health care provider and as part of regular standard of care. Subjects will be followed throughout the duration of the study to assess for study endpoints including MI, stroke, death, and bleeding events (BARC type 2-5). Subjects who experience an endpoint event will continue to be followed throughout the entire study and any subsequent events will also be confirmed and recorded.

8.2 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.2.1 DEFINITION OF ADVERSE EVENTS (AE)

Because this in a non-interventional study and only phlebotomy, a commonly performed procedure, will be performed, an adverse event for this study is defined as any untoward

medical occurrence associated with venipuncture. Given the routine nature of phlebotomy, only those AEs that meet the criteria for serious will be required to be reported and followed to resolution or until considered stable.

For this study, stroke, MI, death, and bleeding events will be reported as endpoint events and will not be considered or reported as adverse events.

8.2.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An AE is considered "serious" if it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

For this study, only SAEs related to phlebotomy will be reported.

8.2.3 CLASSIFICATION OF AN ADVERSE EVENT

8.2.3.1 SEVERITY OF EVENT

The following definitions will be used to describe severity of an SAE:

- **Mild** – Events require minimal or no treatment and do not interfere with the subject's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment.

8.2.3.2 RELATIONSHIP TO STUDY PROCEDURE

All SAEs related to phlebotomy must have their relationship to this procedure assessed by the investigator who will evaluate the event based on temporal relationship and clinical judgment.

- **Related** – The event is known to occur with the study procedure, there is a reasonable possibility that the study procedure caused the SAE, or there is a temporal relationship between the study procedure and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study procedure and the SAE.
- **Not Related** – There is not a reasonable possibility that the study procedure caused the event, there is no temporal relationship between the study procedure and event onset, or an alternate etiology has been established.

8.2.4 SERIOUS ADVERSE EVENT ASSESSMENT AND FOLLOW-UP

All SAEs will be captured in the CRF. Information to be collected includes event description, investigator's assessment of severity, relationship to study procedure, and resolution/stabilization date. All SAEs will be followed to resolution or until considered stable.

8.2.5 SERIOUS ADVERSE EVENT REPORTING

Investigators will monitor subjects for SAEs related to phlebotomy and must immediately report an SAE to the sponsor. In the event a subject experiences a Suspected Unexpected Serious Adverse Reaction (SUSAR) due to venipuncture, the sponsor must notify the Food and Drug Administration (FDA) and all participating investigators of this safety report, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting. Furthermore, the sponsor must also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information.

8.2.6 DATA SAFETY MONITORING

Because of the limited risk of this non-interventional trial, data safety and monitoring will be managed without a formal data safety and monitoring committee. Investigators at each site will report SAEs related to study procedures (only phlebotomy) to the sponsor. The sponsor will bring SAEs to the attention of the co-principal investigators, Dr. Schneider and Dr. Harrington (see Section 10.5). Drs. Schneider and Harrington will be primarily responsible for the oversight of data and safety.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

Specifically, the study hypotheses are:

1. Platelet expression of FcγRIIa is associated with risk of MI, stroke, and death.
2. Incorporating platelet expression of FcγRIIa into existing risk scores improves identification of patients at high risk of MI, stroke, and death.
3. Platelet expression of FcγRIIa is not associated with risk of major bleeding.

9.2 SAMPLE SIZE DETERMINATION

This study is designed to provide a sufficient number of events to build and evaluate a prediction model. The planned sample size of 800 subjects will continue until at least 80 ischemic events have occurred. Under the guideline from Steyerberg of 10 events required per predictor (Steyerberg 2019), this sample size will allow a model with FcγRIIa and up to seven additional predictors to be built. Preliminary results (Schneider 2018) suggest that a 4-fold greater incidence of events will be seen in subjects with high platelet expression of FcγRIIa. For

the primary endpoint, there will be at least 95% power to detect a 2-fold greater incidence of cardiovascular endpoints (MI, stroke, and death) with a two-sided $\alpha<0.01$ assuming 8% of subjects experience the endpoint. With the planned sample size of 800 a hazard ratio (HR) of 1.9 with a p=0.04 (lower bound of the 95% CI of 1.2) can be detected. For the secondary endpoint, there will be 95% power to detect a hazard ratio of 1.93 when comparing bleeding risk in subjects with high compared with low FcγRIIa expression when assuming a two-sided $\alpha=0.05$ and 5% of subjects experience bleeding in the low FcγRIIa expression group.

9.3 POPULATION FOR ANALYSES

All enrolled subjects who are eligible based on the inclusion/exclusion criteria will be included in the population for analysis.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

The planned statistical analyses will be included in a study Statistical Analysis Plan (SAP).

9.4.2 ANALYSIS OF THE PRIMARY OBJECTIVE

Cox proportional hazards models will be fit to compare the risk of the primary endpoint based on platelet expression of FcγRIIa as a continuous variable. Subjects will be followed until their earliest MI, stroke, or death. Patients who do not experience the composite endpoint will be censored at their last telephone follow-up. Time to the primary endpoint will be calculated as days from study enrollment. Models will be adjusted for potential confounders including sex and other subject demographics and medical history collected at baseline. In the pilot study, it was observed that older age, diabetes mellitus, and prior revascularization were associated with higher platelet FcγRIIa expression.

A set of sensitivity analyses will evaluate FcγRIIa as a binary endpoint, which each analysis using a different threshold for classifying FcγRIIa as high or low. In particular, these analyses will explore whether a binary or a continuous measure of FcγRIIa provides greater predictive value, and whether or not a threshold of ≥ 11,000/platelet is optimal.

9.4.3 ANALYSIS OF THE SECONDARY OBJECTIVES

First Secondary Objective: Negative predictive value, positive predictive value, sensitivity, and specificity will be calculated. Prediction will be performed using a Cox regression model with the following predictors: platelet expression of FcγRIIa, sex, age, MVD, CKD, DM, and prior MI. Evaluation of the model's error rates will be based on a leave-one-out cross-validation approach. Additional models will evaluate the performance of the predictive model using a continuous measure of FcγRIIa with the performance of predictive models using various binary measures of FcγRIIa.

Second Secondary Objective: The adjusted Cox proportional hazards model described for the primary objective analysis will be used to compare the bleeding risk as a function of FcγRIIa expression, both as a continuous and as a binary variable.

9.4.4 SAFETY ANALYSES

Not applicable as there is no study intervention.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Descriptive statistics will be used to summarize clinical characteristics.

9.4.6 SUB-GROUP ANALYSES

Not applicable.

9.4.7 SUBSTUDY ANALYSES

Details on the substudy statistical analyses will be summarized in the SAP.

10 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1 INFORMED CONSENT PROCESS

Prior to the start of the study and enrollment of any subjects, investigators must have the IRB's written approval for the protocol and the written informed consent form(s) and any other written information to be provided to the subjects.

Informed consent is a process that is initiated prior to the subject's agreeing to participate in the study and continues throughout the subject's study participation. The subject will be asked to read and review the informed consent document. The investigator will explain the study to the subject and answer any questions. The subject should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The subject will sign the informed consent document prior to any procedures being done specifically for the study. Subjects must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the subject. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the subject undergoes any study-specific procedures. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the sponsor to investigators, study subjects and regulatory authorities. The study may resume if the cause of the temporary suspension has been sufficiently addressed to satisfy the sponsor, IRB and/or regulatory authorities.

10.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their representatives. This confidentiality is extended to cover testing of blood samples in addition to the subject's clinical information. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The sponsor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital). The clinical study site will permit access to such records upon request.

The subject's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study subject research data will be used for statistical analysis and scientific reporting, however this will not include the subject's contact or personal identifying information. Individual subjects and their research data will be identified by a unique study identification number.

After informed consent is obtained, each subject will be assigned a unique identifier. Clinical characteristics and biochemical results will be associated with the unique identifier. The association between the subject identity and the unique identifier will be kept in a secure file. Access to that information will be restricted to study personnel obtaining the information from the subject and key study personnel. Blood and derived specimens will be labeled with the unique identifier only.

Additionally, all study sites operate in full compliance with Health Insurance Portability and Accountability Act (HIPAA) regulations.

10.4 USE OF BLOOD SAMPLES

Blood samples taken for this study will be prepared, labeled, and shipped to the central laboratory for testing (see separate Laboratory Sample Manual for details). Blood, derived specimens, and biochemical results will be labeled and associated with the unique identifier only. Blood and derived specimens will be consumed during the assay process and any remaining samples will be appropriately destroyed at the end of the study. Samples will be assayed at a core laboratory and only study personnel will have access to the samples. After all assays have been completed and results confirmed, any remaining sample will be destroyed in accordance with the institutional biohazard disposal process. Samples will not be stored for use or testing beyond that described for this study. During the conduct of the study, an individual subject can choose to withdraw consent to have their blood sample tested, however, withdrawal of consent may not be possible after the sample has been tested.

10.5 PRINCIPAL CO-INVESTIGATORS AND STUDY GOVERANCE

The overall study Principal Co-Investigators are David J. Schneider, MD and Robert A. Harrington, MD (contact information below).

David J. Schneider, MD
Professor of Medicine
Medical Director of Cardiovascular Services
Director of Cardiovascular Research Institute
University of Vermont/University of Vermont Health Network
Burlington, VT 05401

Robert A. Harrington, MD
Arthur L. Bloomfield Professor of Medicine
Chair, Department of Medicine
Stanford University
Mail: 300 Pasteur Drive, S-102
MC: 5110
Stanford, CA 94305

Drs. Schneider and Harrington will be primarily responsible for the oversight of data and safety.

There are no leadership committees and no Medical Monitor for this study.

10.6 CLINICAL MONITORING

Remote, centralized, and targeted data review will be conducted to ensure that the reported clinical data are accurate and complete, and that the conduct of the study is in compliance with the currently approved protocol/amendment(s). CRFs will be submitted periodically to the sponsor for review, database entry and endpoint event tracking. Source documents of endpoint events will be collected by the site and submitted for review by the sponsor. The investigator will confirm the endpoint event and there will be no adjudication by a separate committee.

10.7 QUALITY CONTROL

Quality control (QC) procedures will be implemented beginning with built in checks at data entry and in routine reports and QC checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

10.8 DATA COLLECTION AND RECORD RETENTION

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. Source data will be captured in the subject's medical record, either electronic or hard copy in a neat, legible manner to ensure accurate interpretation of data.

Data recorded in the CRF derived from source documents should be consistent with the data recorded on the source documents. For subjects who experience an endpoint event, source documentation to confirm the event will be sent to the sponsor with any subject personal identifiable information redacted.

Sites will enter data directly into REDCap (Research Electronic Data Capture) Cloud, a secure web-based application designed to support data capture for research studies (Harris 2009). Data will be entered into an electronic CRF (eCRF) using REDCap Cloud and transmitted to the sponsor on a routine basis. REDCap Cloud databases are routinely backed-up and address the risk of storing sensitive clinical research data on the user's computer.

Study documents should be retained for a minimum of 2 years after the completion of the study. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable.

10.9 PROTOCOL DEVIATIONS

For this study, the only deviation to the protocol is if any of the inclusion are not met or if any of the exclusion criteria are met.

10.10 PUBLICATION POLICY

There is a commitment to submit results of this study for publication within 18 months after database lock.

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12 APPENDIX 1 – STUDY ENDPOINT EVENT DEFINITIONS

Definition for Acute Myocardial Infarction (types 1, 2 and 3 MI) [Thygesen 2018]

The term acute myocardial infarction should be used when there is acute myocardial injury with clinical evidence of acute myocardial ischemia and with detection of a rise and/or fall of cTn values with at least 1 value above the 99th percentile URL and at least 1 of the following:

- Symptoms of myocardial ischemia;
- New ischemic ECG changes;
- Development of pathological Q waves;
- Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischemic etiology;
- Identification of a coronary thrombus by angiography or autopsy (not for types 2 or 3 MIs).

Postmortem demonstration of acute atherothrombosis in the artery supplying the infarcted myocardium meets criteria for type 1 MI. Evidence of an imbalance between myocardial oxygen supply and demand unrelated to acute atherothrombosis meets criteria for type 2 MI. Cardiac death in patients with symptoms suggestive of myocardial ischemia and presumed new ischemic ECG changes before cTn values become available or abnormal meets criteria for type 3 MI.

Definition for Stroke [Sacco 2013]

The term stroke should be used when there is focal ischemia within the perfusion territory of an artery that is stenosed or occluded, and cell death is localized to this region. In focal cerebral ischemia, cell death is maximal in the ischemic focus and may extend to the penumbra, with all cellular elements including both neurons and supportive cells affected. Clinical evidence of stroke includes both of the following:

- Symptoms of focal ischemia (e.g., unilateral weakness of an arm or leg) and
- Imaging evidence (CT or MRI) of CNS infarction

Bleeding Academic Research Consortium (BARC) Definition for Bleeding [Mehran 2011]

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 healthcare professional; may include episodes leading to self-discontinuation of medical therapy by the patient without consulting a healthcare professional

Type 2: any overt, actionable sign of hemorrhage (eg, 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: (1) requiring nonsurgical, medical intervention by a healthcare professional, (2) leading to hospitalization or increased level of care, or (3) prompting evaluation

Type 3:

Type 3a:

Overt bleeding plus hemoglobin drop of 3 to <5 g/dL* (provided hemoglobin drop is related to bleed)

Any transfusion with overt bleeding

Type 3b:

Overt bleeding plus hemoglobin drop ≥5 g/dL* (provided hemoglobin drop is related to bleed)

Cardiac tamponade

Bleeding requiring surgical intervention for control (excluding dental/nasal/skin/hemorrhoid)

Bleeding requiring intravenous vasoactive agents

Type 3c:

Intracranial hemorrhage (does not include microbleeds or hemorrhagic transformation, does include intraspinal)

Subcategories confirmed by autopsy or imaging or lumbar puncture

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 ≥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

Note: CABG indicates coronary artery bypass graft. Platelet transfusions should be recorded and reported but are not included in these definitions until further information is obtained about the relationship to outcomes. If a CABG-related bleed is not adjudicated as at least a type 3 severity event, it will be classified as not a bleeding event. If a bleeding event occurs with a clear temporal relationship to CABG (ie, within a 48-h time frame) but does not meet type 4 severity criteria, it will be classified as not a bleeding event.

*Corrected for transfusion (1 U packed red blood cells or 1 U whole blood=1 g/dL hemoglobin).
†Cell saver products are not counted.

Abbreviations: CNS, central nervous system; CT, computed tomography; cTn, cardiac troponin; ECG, electrocardiogram; g/dL, grams/deciliter; h, hour; MI, myocardial infarction; MRI, magnetic resonance imaging; U, units; URL, upper reference limit.

INVESTIGATOR AGREEMENT

I have read this protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study intervention, the conduct of the study, and the obligations of confidentiality.

Principal (Site) Investigator:

Name (typed or printed): _____

Institution and Address: _____

_____

_____

_____

Telephone Number: _____

Signature: _____ Date: _____

(Day Month Year)

Sponsor's Responsible Officer:

Name (typed or printed): Peter M. DiBattiste, MD

Company: Prolocor, Inc

Signature: Peter M. DiBattiste, M.D. Digitally signed by Peter M. DiBattiste, M.D. Date: 2022.02.03 21:34:03 -05'00' Date: _____

(Day Month Year)