Evolocumab in Patients With Acute MI
NCT04082442
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JHM IRB - eForm A – Protocol

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1. Abstract

a. Provide no more than a one page research abstract briefly stating the problem, the research hypothesis, and the importance of the research.

Despite aggressive early intervention and current secondary prevention strategies, hundreds of thousands of patients who survive hospitalization for acute coronary syndrome (ACS) experience subsequent unfavorable outcomes, including recurrent ischemic events and unfavorable cardiac remodeling associated with progressive left ventricular dysfunction and congestive heart failure. Vascular and myocardial inflammation are significantly increased in ACS patients, are closely correlated to LDL-C levels, and are associated with these adverse consequences in the post-ACS patient population. Inflammation may also play a role in increased platelet aggregation one of the responsible mechanisms for the index and for recurrent coronary thrombotic events. Serum proprotein convertase subtilisin/kerin type 9 (PCSK9) levels are also increased in ACS, may raise LDL-C, and our pre-clinical studies indicate that PCSK9 is also a potent inducer of vascular inflammation. The addition of the PCSK9 antibody evolocumab, currently approved to lower LDL-C in certain patient populations, to current medical therapies may be of particular benefit in an important subset of ACS patients, those with ST-elevation and non-ST-elevation myocardial infarction by markedly reducing LCL-C, stabilizing vulnerable plaque, and limiting inflammation-associated myocardial cell loss and resultant dysfunction.

Hypothesis: In a placebo-controlled, randomized double blind trial, the addition of the PCSK9 antibody evolocumab to standard care in ST-elevation (STEMI) and non-ST elevation (NSTEMI) patients (1) decreases LDL-C during hospitalization and at 25-30 days, (2) decreases vascular/plaque and myocardial inflammation as assessed by PET scanning at 25-30 days, and improves (3) serum markers of endothelial function and of platelet function at hospital discharge, and at 25-30 days (4) echocardiographic at 30 days and, in a subset of patients (5) magnetic resonance imaging (MRI) assessment of left ventricular function at six months.

This is the first PCSK9 inhibitor trial which examines these outcomes in the NSTEMI and STEMI patient population. It will provide valuable data on the extent and time course of LDL-C reduction as well as the impact of PCSK9 inhibition and, we believe, of changes in inflammation on serum

pro-inflammatory markers and on imaging assessment of vascular and myocardial inflammation, all of which may significantly impact important clinical outcomes in this high risk patient cohort.[1]

Observation Study:

To further assess the trajectory of Lp(a) and other inflammatory mediators and markers in patients with acute myocardial infarction we are requesting permission to obtain samples from an additional 100 patients not enrolled in the current protocol, and not undergoing any research procedures other than venipuncture. They will be obtained from the time of early hospital evaluation, during hospitalization, and at 30 days. The first sample may that obtained clinically and sent to the laboratory during the early hospital evaluation, but not used by the laboratory (sometimes termed "remnant sample").

Rationale for the observation study:

The motivation for the study is two-fold. First, to more clearly define the trajectory of Lp(a) and other inflammatory serum and plasma markers/mediators in ACS patients and more specifically to obtain measures at the time of first presentation (e.g. in the Emergency Department) using any "remnant" samples obtained at that time. Second, to perform detailed proteomics on the samples using the sophisticated technology at Proteomics Bioanalytics. The company has an outstanding scientific publication record, and this is an opportunity for us to establish a relationship with them. The data will allow us to examine the importance of proteomic measures of potential inflammatory markers and mediators, which is a prime focus of my basic studies.

Although, as previously noted, Lp(a) is recognized as a risk factor for ACS patients, there has not until very recently, been an intervention specifically directed to that risk factor. Amgen (and Novartis) developed an intervention which works at the RNA-to-protein step in Lp(a) synthesis. Amgen is preparing a large phase 3 trial and may incorporate our data in a determination as to whether to include an "ACS arm" in their study.

2. Objectives (include all primary and secondary objectives): To conduct a placebo-controlled randomized, double blind study of the effect of the addition of evolocumab to standard care in NSTEMI and STEMI patients

2.1 Primary (for the intervention study group)
The primary objectives are the difference, in the evolocumab and placebo groups, in the mean % change in LDL-C from pre-randomization to 25-30 days.

Primary (for the observation study group)
The time course of inflammatory markers and mediators in those with acute myocardial infarction.

2.2 Secondary (for the intervention study group)
The secondary objectives are to compare in the evolocumab and placebo groups:

a. Serial serum markers of inflammation, platelet and endothelial cell function, lipids, and myocardial injury, and left ventricular function which will be collected at pre-randomization, during hospitalization, at hospital discharge and at 25-30 days, 6 months and 12 months follow up. In those who are discharged before day 3, and those who are unwilling or unable to return to the hospital, additional samples may be obtained in the out of hospital setting.

The time points of comparison are pre-randomization, during hospitalization, at hospital discharge, and any obtained in the outpatient setting including the 30 days, 6 months, and 12 months samples.

i. PCSK-9
ii. Soluble LOX-1
iii. IL-6, IL-10, IL-12
iv. hsCRP
v. platelet function
vi. LDL-Cholesterol
vii. HDL-Cholesterol
viii. Triglycerides
ix. Total Cholesterol
x. Apolipoprotein B
xi. Lipoprotein (a)
xii. Troponin
xiii. Pro-BNP
xiv. HbA1c
xv. Insulin

b. The change between hospitalization and thirty day of:
   i. PET-FDG assessed myocardial inflammation and vascular inflammation in the most diseased segment of aorta or carotid artery. For this outcome, separate analyses will also be performed in those who did and did not undergo the initial PET scan within 48 hours of study drug administration.
   ii. Echo assessed regional wall motion in the infarct territory
   iii. Echo assessed global LV function
   iv. Echo assessed LV systolic and diastolic volumes

   The times of collection will be at early hospitalization and 30 days. The time point of comparison will be at 30 days

c. At thirty days:
   i. Comparison of PET-FDG assessment of myocardial inflammation and vascular inflammation in the evolocumab versus placebo group as described in 2.2. b i
   ii. Echo assessed as described in 2.2. b ii-iv
   iii. Angina class
   iv. New York Heart Association functional class
   v. Serum markers as described in 2.2. a

d. At six months
   i. Angina class
   ii. New York Heart Association functional class
   iii. Echo assessment as described in 2.2. b ii-iv
   In a subset of participants: The change between early infarction period and six months assessments of Cardiac MRI-assessed adverse remodeling, e.g. LV function and volumes, infarct size and scar quantification

e. At 12 months
   Serum markers as described in 2.2 a

Secondary (for the observation study group): The change in inflammatory markers and mediators between early hospitalization, during hospitalization, and at 30 days.

2.3 Exploratory

The exploratory basic science outcome is: Direct comparison of endothelial cell function in cells harvested from intravascular equipment or directly from patient serum in the evolocumab- and placebo-randomized patient groups.

The exploratory imaging outcomes are: To compare PET assessed myocardial and vascular inflammation in the evolocumab and placebo groups on the exams obtained during the hospitalization.

3. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

According to the latest AHA fact sheet, over 400,000 Americans suffered a NSTEMI or a STEMI in 2017 [2]. Despite aggressive risk factor modification in these patients, recurrent ischemic events are frequent and are related to plaque burden and instability, platelet aggregation, and ongoing inflammation [3]. These patients have a high risk of short-term unfavorable outcomes, including mortality, and therefore may significantly benefit from an early intervention which would significantly decrease those risks [4]. Previous studies indicated an inflammatory environment in the acute infarct setting as indexed by high levels of hs-CRP, total white blood cell count and IL-6 [5]. Inflammation contributes to many of the characteristics of those plaques implicated in the pathogenesis of recurrent ischemic events [6]. The pathways which promote fibrous cap rupture involve inflammation [7] and excess lipid accumulation In a large cohort of patients suffering a first myocardial infarction, new heart failure is associated with an increase in index hospitalization mortality and in those who develop heart failure following discharge. Short- and long- term mortality are significantly greater in those NSTEMI and STEMI patients who develop heart failure [8]. Inflammatory mediators are also activated in heart failure after acute myocardial infarction and are associated with progressive left ventricular dysfunction [9].

Evolocumab is a monoclonal antibody that inhibits PCSK9 and is FDA-approved as an adjunct to diet and maximally tolerated statins for use in certain patients to further significantly lower LDL-cholesterol level. In a large randomized clinical trial in patients with stable atherosclerotic cardiovascular disease (a median of 3 years after an event) and LDL cholesterol levels of 70 mg per deciliter or higher who were receiving statin therapy, the addition of evolocumab significantly reduced the risk of cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina, or coronary revascularization during a median follow-up of 2.2 years [10]. In this study, the median LDL cholesterol on statin therapy was 92 mg / dL and the addition of evolocumab reduced LDL cholesterol by 59% to a median of 30 mg / dL. Little is known regarding other potential additional benefits of PCSK9 inhibition in the setting of ACS.

ACS is associated with a marked increase of serum PCSK9 that likely has an important pathophysiologic role in mediating vascular and myocardial inflammation, leading to plaque vulnerability of both culprit and non-culprit coronary vessels as well as adverse myocardial remodeling and resulting contractile dysfunction. Growing evidence emphasizes a potential role of PCSK9 antibodies beyond their LDL-cholesterol lowering capacity (pleiotropic effects), which are likely beneficial in managing NSTEMI and STEMI [11]. The Ottawa Heart Genomics Study and the Emory Cardiovascular Biobank both demonstrated an association between elevated PCSK9 levels and adverse acute and long-term events in patients with ACS. PCSK9 levels are markedly elevated during an ACS, and in one study were related to the composite endpoint of death and recurrent ACS at 1-year follow-up.[12] Additional data indicate that elevated PCSK9 adversely

affects coronary plaques through several pathways, including pro-inflammatory LDL oxidation and direct modification of plaque composition [13-15]. Additionally, chronically elevated PCSK9 levels are associated with progression of atherosclerosis [12]. Finally, a recent study suggests that increased PCSK9 levels are associated with higher platelet reactivity and are a possible predictor of ischemic events in ACS patients [16].

Over the past few decades, our understanding of the vascular biology of atherogenesis and its clinical presentations have advanced significantly. It is now apparent that inflammatory processes play a key role not only in the initiation and progression of ASCVD but also in the clinical events associated with instability of already established atherosclerotic plaques [17-19]. Traditional and novel cardiovascular risk factors trigger a chronic inflammatory process, which is accompanied by loss of vasodilatory and antithrombotic properties of the vascular endothelium [20-22]. Clinical studies show that this emerging biology of inflammation in atherosclerosis applies directly to human pathology [23]. Elevated inflammatory markers predict outcomes of ACS patients independently of the extent of myocardial damage [23]. In addition, low-grade chronic inflammation, as indicated by levels of the inflammatory marker hsCRP, prospectively independently defines risk of atherosclerotic complications, thus adding to the prognostic information provided by traditional risk factors [24, 25]. Moreover, certain treatments that reduce coronary risk also limit inflammation [26, 27].

Identifying reliable endothelial cell (EC) function markers for *in vivo* testing remains a challenge. In this clinical trial, we propose to utilize the emerging EC function marker sLOX1. The LOX1 receptor is an endothelial receptor for atherogenic oxidized-LDL and is implicated in the pathogenesis of atherosclerosis [28]. In addition, nuclear imaging studies detecting radiolabeled LOX1 report significantly higher accumulation of LOX1 in grade IV atheroma than in more stable lesions [29]. It was recently reported that LOX1 is converted to soluble molecules by proteolytic cleavage at the membrane proximal site of the extracellular domain. In the same study, EC activation by pro-inflammatory stimuli, e.g. TNF-a, led to cell-surface expression of LOX-1 which precedes soluble LOX-1 production [30]. sLOX1 is also proposed as a marker of plaque instability in ACS patients [31]. Finally, persistently elevated sLOX1 levels in ACS patients are linked to adverse clinical outcomes [32]. Based on the scientific evidence, we believe that sLOX1 is a suitable marker for endothelial dysfunction and possibly plaque instability in the setting of ACS and STEMI.

In this study, we will utilize non-invasive PET imaging to assess vascular/plaque and myocardial inflammation. A recent review article nicely summarizes the utility of PET in the setting of ACS [33]. The strength of PET-guided imaging in this setting is the identification of activated inflammatory cells in the atherosclerotic plaque, providing important information regarding the mechanism of vascular instability. Furthermore, longitudinal PET studies can assess the effectiveness of potentially novel anti-inflammatory therapies, such as evolocumab. Initial validation of FDG-PET imaging in the setting of ACS was performed in mice [34], but other reports also provide proof of feasibility in large animals and humans [35, 36]. Ripschler et al. subsequently demonstrated that the extent of FDG-derived myocardial inflammation early after myocardial infarction correlated with infarct size and systemic inflammatory markers at that time and with left ventricular ejection fraction and cardiac volumes at 6-9 month follow-up [37].

The multimodality dal-PLAQUE imaging study successfully utilized FDG-PET to assess plaque inflammation and burden in patients with a high risk of coronary heart disease [38]. A subsequent study reported a functional alteration of the plaque, characterized by activation of resident inflammatory cells, which can cause plaque instability and occlusive thrombus formation [39]. Since persistent activation of adaptive immunity in patients with unstable angina is linked to

coronary instability [40], we propose to evaluate the impact of evolocumab on the extent and degree of vascular inflammation as well in our study cohort.

4. Study Procedures (for the intervention study group)

a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).

This is a phase 2, double-blind, randomized, placebo controlled clinical trial of 420 mg evolocumab administered subcutaneously using an autoinjector/pen in NSTEMI and STEMI patients during early hospitalization at the Johns Hopkins Hospital and the Johns Hopkins Bayview Medical Center. The placebo and evolocumab will be given exactly the same way with the same Amgen provided device. All participants will receive standard care for NSTEMI and STEMI, including, as appropriate, high dose statin, aspirin, beta blocker, an angiotensin converting enzyme inhibitor or angiotensin receptor blocker, as well as angiography and reperfusion. High dose statin therapy consists of atorvastatin 40mg or 80mg or rosuvastatin 20mg or 40mg per the most recent ACC/AHA guidelines. [41] After signing informed consent, subjects will be randomized to receive one dose of evolocumab or matching placebo subcutaneously. Research blood samples for lipid profile, endothelial function, and inflammatory markers, and PET imaging assessment of myocardial and vascular inflammation as well as any clinically indicated echocardiographic studies for left ventricular function will also be obtained. Additionally, in a subset of patients we will obtain cardiac MRI studies. An independent Data and Safety Monitoring Committee will review all potential side effects and clinical outcomes during the six months of follow-up.

FDG/PET scans are not performed at Hopkins Bayview. If participants enrolled at that site are transferred to Hopkins Hospital for clinical care, and the scan can be performed during that hospitalization, it will be done so. For other patients, i.e. those discharged home from Bayview, or Hopkins patients who could not undergo the scan during their admission because of scheduling issues, the first scan will be performed on an outpatient basis. In those instances a car service will be arranged to transfer the participants to and from JHH and they will have three outpatient visits, for the initial and the follow-up 30 day scans, and for the six-month visit.

After hospital discharge, we will send a letter to the participant's physician(s) to inform them that the participant was enrolled in the study and that lipid values during the 4-5-week period after study drug administration may reflect the impact of evolovumab if the participant is randomized to that agent. The letter is attached in the supplemental study documents.

At the 25-30-day follow-up visit we will perform a physical exam and obtain a history of any clinical events including adverse events. We will perform a second FDG-PET study to assess vascular and myocardial; inflammation. Additionally, we will assess lipid profile, and serum markers of inflammation and of endothelial and platelet function as outlined in 2.2 a.

At approximately 3 month we will contact the participant by phone to learn of any adverse events. If the participant was admitted to the Emergency Department or Hospital we will obtain permission to obtain medical records.

At the 6-month follow-up visit will perform a physical exam and obtain a history of any clinical events including adverse events. Additionally, we will assess lipid profile and serum markers of inflammation and endothelial function as outlined in 2.2 a. In the subset of participants for

whom we have MRI studies during the early infarction period, we will obtain a second cardiac MRI studies to assess adverse remodeling.

At approximately 9 months we will contact the participant by phone to learn of any serious adverse events. If the participant was admitted to the Emergency Department or Hospital we will obtain permission to obtain medical records.

At 12-month follow-up visit we will assess any serious adverse events, lipid profile and serum markers of inflammation and endothelial function as outlined in 2.2 a.

All above are research studies with the exception of the clinical echocardiogram during the initial hospitalization.

Study Procedures (for the observation study group)
Three venipunctures; obtained during early hospitalization, during hospitalization, and at 30 days.

COVID-19 Specific Accommodations
Due to the Coronavirus 2019 pandemic, additional steps will be taken for the safety and wellness of study participants and staff.

Pre-Visit Considerations:
- Participants will be advised about visitor restrictions, face coverings policy, need for physical distancing and hand hygiene per published JHH guidelines.
- Participants will receive a COVID Risk Sheet during consent process or before their research visit if they are already consented. This was downloaded and is attached.
- Participants will receive COVID risk screening conducted by the study team at the time they are given an appointment and the day before or before leaving home to come to the appointment. We will use the most recent published JHMI standards.
- If applicable, we will record the following over the phone prior to the study visits:
    - Inclusion and exclusion criteria –prior to initial visit
    - Demographics – prior to initial visit
    - Medical history – prior to all study visits
    - Adverse events – prior to all study visits
    - Serious adverse events – prior to all study visits
    - Concomitant therapies – prior to all study visits
- As much as possible, the staff will use ECGs and vitals taken clinically.

Visit Considerations:
- All providers and staff will wear appropriate face coverings: surgical facemasks and face shields.
- The study team has obtained JHH approved PPE.
- Participants will wear face covering during their research visit.
- We will adhere to 6-foot distance between participants and staff or between staff and other staff – when feasible. Number of staff members interacting with the participant will be limited as much as possible.
- No visitors for adult participants will be permitted.
- Upon arrival, study staff will meet participants at the entrance and walk them to the front desk where they again receive COVID screening.
- Visit procedures (PET scan and echo) will be scheduled to minimize waiting time between each procedure. While the participant waits the scan to begin, study staff will draw blood

from the IV and perform an ECG. Otherwise, the participant will wait alone in the room which is video monitored.
- Participants must be fasted for the PET scan. The study team will provide a variety of snacks and small food items to break their fast. Participants will not need to eat in the cafeteria.

Room Cleaning:

• Rooms will be cleaned by research staff with Sani - Cloth wipes after each patient. (Desk, exam table-if used, chairs.) Computer surfaces will be wiped (keyboard, mouse, screen) if used.

b. Study duration and number of study visits required of research participants.

The study duration for each participant is 12 months and there are three study visits required.

c. Blinding, including justification for blinding or not blinding the trial, if applicable.

The investigators and participants are blinded to study drug assignment. Blinding is necessary to avoid potential bias in data gathering, analysis, and interpretation. The Hopkins Investigational Pharmacy will have access to study drug assignment if there is a clinical need for the subjects' treating physicians to be aware of assignment.

d. Justification of why participants will not receive routine care or will have current therapy stopped.

All participants will receive routine care and will not have current therapy stopped.

e. Justification for inclusion of a placebo or non-treatment group.

Inclusion of a placebo, comparator group is necessary to best determine the safety and efficacy of evolocumab in NSTEMI and STEMI patients. As noted above, evolocumab is not considered "treatment" for this patient population and hence we do not believe there is a "non-treatment" group, or ethical concern by inclusion of a placebo group.

f. Definition of treatment failure or participant removal criteria.

There is no defined "treatment failure." Outcomes of those randomized to evolocumab or placebo will be collected to determine whether the "treatment" was or was not successful in terms of the safety and efficacy outcomes.

Participants are volunteers and may decline continued participation and if so will be removed from any further study procedures. In addition, those who undergo unanticipated coronary bypass surgery will not undergo the second PET study as cardiopulmonary bypass itself is associated with increased inflammation and therefore would confound interpretation of the inflammatory outcomes. We will gather the lipid outcomes in these individuals.

<u>Early stopping rules:</u>

The study will be stopped early on the recommendation of the Data Safety and Monitoring Committee or if there is any new published information regarding the safety of the study drug or the study procedures.

g. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

Participants will only receive one dose of the study drug on the day of enrollment and thus will not be receiving any study related therapy when the study ends or if the participant's participation in the study ends prematurely.

Study Procedures (for the observation study group)

a. Blood draws:
    i. Participants will undergo 4 blood draws of about 3-4 tablespoons each to measure lipoprotein (a) [Lp(a)], cholesterol levels, and thrombotic and inflammatory markers and mediators. . The first blood draw will be at the time the participant presents to the hospital, followed by an additional blood draw during the inpatient stay, a blood draw at a 30-day follow-up visit and a final blood draw at a 6 months follow-up visit.
b. Obtaining remnant samples from blood draws in the ED:
    i. After a patient agrees to participate and signs the ICF, we will access the JHH remnant sample repository to obtain remnant blood from the earliest timepoint at hospital presentation. We will work with the JHBMC Pathology Department to locate and retrieve the samples for analysis of the proposed biomarkers.

5. Inclusion/Exclusion Criteria

5.1 Inclusion Criteria (for the intervention study group)

a. Age 25 to 90 years.
b. Non-ST-elevation and ST elevation myocardial infarction, with compatible symptoms and ECG changes.
c. Permission of attending physician.
d. Ability to understand the risk, benefits, and alternatives of participation.
e. Ability to administer study drug within one week of hospital presentation.

5.2 Exclusion Criteria

a. Scheduled for cardiac surgery.
b. Current treatment with a PCSK9 antibody.
c. Latex allergy
d. Previous adverse reaction to monoclonal antibodies
e. Known sensitivity to evolocumab
f. Non-English speaking
g. Current participation in an intervention clinical trial without the permission of that trial's principal investigator or in which the study procedures or outcomes of that trial would be impacted by those of this study or vice versa.
h. Female of childbearing potential. This is a female subject who has not used acceptable method(s) of birth control (see below) for at least one month prior to screening, unless the

subject is sterilized or postmenopausal. Menopause is defined as: 12 months of spontaneous and continuous amenorrhea in a female ≥ 55 year of age.

- Acceptable method(s) of birth control definition: One highly effective method (methods that can achieve a failure rate of less than 1% per year when used consistently and correctly)

i. Combined hormonal (estrogen and progestogen) contraception associated with inhibition of ovulation (oral, intravaginal, transdermal)
ii. Progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable)
iii. Intrauterine device (IUD)
iv. Intrauterine hormone-releasing system (IUS)
v. Bilateral tubal occlusion
vi. Vasectomized partner
vii. Sexual abstinence

i. Subject likely not to be able to complete all protocol-related study visits or procedures.
j. For the cardiac MRI studies:
   i. Creatinine clearance, CrCl, <45 ml/min/1.73m^2
   ii. MRI contrast allergy
   iii. Contraindications to MRI, including any implanted or history of known or potential placement of ferromagnetic material and claustrophobia.

6. Inclusion/Exclusion Criteria (for the observation study group)

7.

5.1 Inclusion Criteria

a. Age 25 to 90 years.
b. Non-ST-elevation and ST elevation myocardial infarction, with compatible symptoms and ECG changes.
c. Permission of attending physician.
d. Ability to understand the risk, benefits, and alternatives of participation.

7.2 Exclusion Criteria

c. Scheduled for cardiac surgery.
d. Current treatment with a PCSK9 antibody.
e. Subject likely not to be able to complete all protocol-related study visits or procedures.

8. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.

The drug evolocumab was chosen because of basic laboratory studies indicating that PCSK9 mediates inflammation-induced endothelial cell dysfunction, a clinical study I participated in indicating that the PCSK9 antibody improves coronary endothelial function, and because of its safety profile. Evolocumab also improves lipid profile in patients already on statin therapy. [10] The dose was chosen based on that approved by the FDA for clinical use in patients with stable coronary disease, available in the United States and reduces LDL cholesterol. [42]

b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

Evolocumab is an FDA approved medication that will be administered for a non-FDA approved indication, patients with NSTEMI and STEMI. This is justified based on clinical and pre-clinical data indicating that vascular and myocardial inflammation are increased in NSTEMI and STEMI patients, that inflammation is associated with adverse outcomes in this patient group, that in stable CAD patients' anti-inflammatory interventions improve outcomes, and that evolocumab has anti-inflammatory properties. The dose and route of administration are the same as that administered for FDA approved indications and placebo-matching autopen injector systems will be provided by Amgen.

We are applying for an exemption from the FDA such that an IND is not needed for the proposed mechanistic study in NSTEMI and STEMI patients. Safety information on the use of these drugs is detailed above.

An IND exemption request will be submitted to the FDA.

9. Study Statistics (intervention study)

Primary:

For the intervention study group: Difference in the mean % change in LDL-C from pre-randomization to 25-30 days.

For the observation study group: Trajectory of Lp(a) in patients with acute myocardial infarction

Secondary (for the intervention study group):

1) LDL-C at 25-30 days in the two groups.

2) Change in vascular inflammation from the FDG-PET scan obtained during the early post-infarct period and the 25-30 day FDG-PET scan in the evolocumab and placebo groups. Vascular inflammation will be assessed in the average of the TBR (target to background) of the MDS (of most diseased segments) of the carotid artery or aorta in the evolocumab vs. placebo groups. The target to blood pool ratio or TBR is the ratio of the mean activity in the vessel to the venous blood pool. [43]

3) Change in myocardial inflammation from the FDG-PET scan obtained during the index hospitalization and the thirty-day FDG-PET scan in the evolocumab and placebo groups.

4) Difference in the FDG-PET assessment of vascular inflammation in the MDS of carotid artery or aorta in the evolocumab versus placebo groups at 30 days.

5) Difference in the FDG-PET assessment of myocardial inflammation at 25-30 days in the evolocumab and placebo groups.

5) Serum markers of inflammation and of endothelial and platelet function in the two randomized groups at 25-30 days.

6) Change in serum markers of inflammation and of endothelial and platelet function in the two randomized groups from the index hospitalization to 25-30 days.

Secondary (for the observation study group)

1. Change in levels of inflammatory markers and mediators between the initial hospitalization, during hospitalization and 30-day follow-up

Sample Size Considerations (for the intervention study group)

We are providing power considerations for the primary as well as key secondary endpoints (see Tables 1-3).

Primary endpoints:
For calculation of power for the co-primary endpoint of LDL-C, we anticipate median baseline presentation LDL-C of 110 (90 – 130) mg/dL in our patient population. This number is informed by prior ACS trials as well as local experience with the STEMI population treated at the Johns Hopkins Hospital (with approximately 25%-35% of the population already on baseline statin therapy). We expect this LDL-C value to be reduced by approximately 50% by high intensity statin therapy in the standard treatment arm (i.e. to an LDL-C of 55 (40- 70) mg/dL at 30 days). We anticipate a further 50% lowering of LDL-C at 30 days after the administration of evolocumab (i.e. to an LDL-C of 28 (14 – 42) mg/dL. As is shown below (Table 1), using a one-sided p-value of 0.05, the study has nearly 100% power to detect this difference, and will have 80% power to detect an absolute LDL-C reduction of only 13.5 mg/dL (24% reduction in LDL-C at 30 days). Additionally, Table 2 presents the expected mean ± SD baseline and 30-day LDL-C levels and the expected percent changes in the mean LDL-C in the placebo and evolocumab arms.

Table 1:

N in each group	**Median (IQR) LDL-C in ACS Population at baseline**	**Anticipated Percent LDL-C Reduction in the Standard of Care Arm**	**Estimated Power to Detect a 50% Additional LDL Lowering**	**Detectable Point Estimate at >80% Power**
N = 50	110 (90-130)	50% (40 – 60%)	99+%	-13.5 mg/dL

Table 2: Expected mean ± SD LDL-C levels at baseline and thirty-days and the percent change from baseline to 30 days in the placebo and evolocumab groups.

Groups:		**LDL-C, mg/dl**		**% change from baseline, mean (SD)**
		Baseline, mean (SD)	**30 day, mean (SD)**	
High dose statin	**+Placebo**	110 (±14)	55 (±11)	-50 (±12)
	+Evolocumab		28 (±10)	-75 (±14)

For the co-primary FDG-PET assessed myocardial inflammation endpoint, appropriate data necessary for power calculations are currently unavailable. With 50 patients in each arm, applying the overall mean (SD) of 35.4 (18.1) from Ripschler et al [38] as a baseline estimate, we will have 80% power to detect a 30 % difference in FDG-PET assessed myocardial inflammation between the evolocumab and placebo groups.

Secondary Endpoint:
Previously published clinically relevant, drug-induced TBR changes are reported in the range of 5%-15%. [44] The power calculations for a 10% and 15% TBR difference based on a sample size of 50 patients per study arm are outlined in Table 3 below. Table 1 uses a 1-sided p-value of 0.05, under the hypothesis that evolocumab will lower inflammation and will not raise it,

consistent with the approach used in the Dal-PLAQUE study. Calculations are performed using non-parametric statistics accounting for the possibility of non-normally distributed TBR data.

Tables 3: Power calculations using a 1-sided p-value, i.e. only assumes that PCSK9 inhibition will lower TBR in the most diseased segment (MDS) and will not raise TBR.

Table 3	**N in each group**	**Median (IQR) TBR in STEMI Population at 30 days**	**Estimated Power at 10% & 15% TBR Difference**	**Detectable Point Estimate at 70% & 80% Power**
Aorta MDS	N = 50	3.2 (2.7 – 4.0)	88% 99%	-8.1% -9.1%
Carotid MDS	N = 50	2.5 (2.1 – 3.0)	97% 99%	-6.4% -7.2%

The table above shows good to excellent power to demonstrate a difference of 10%, and an excellent power to show a difference of 15% in the primary outcome.

Mean and standard deviations of anticipated TBR in STEMI population at 30-days:

- Aorta MDS: 3.3 ± 0.6
- Carotid artery MDS: 2.6 ± 0.4

Since the timepoint of study drug administration may vary between participants hospitalized at JHH and Bayview, we will create a co-variable to document the time period between hospitalization and study drug administration and adjust our analysis accordingly.

Sample Size Considerations (for the observation study group)

Analysis of Lp(a) in 37 patients in the placebo group of the intervention study indicates an increase in Lp(a) (median, IQR) from 64 [41,187] to 82 [37,265] nmol/L from that obtained during the hospitalization and at 30 days. We would like to obtain an earlier value and correlate the findings with other inflammatory markers and mediators. Given the findings in the above analysis, we believe results in 100 additional patients would provide an adequate sample size.

a. Early stopping rules.

The DSMB will perform any interim analysis they require to monitor patient safety and provide reports advising continuation of the study.

10. Study Statistics (observation study):

Statistical Approach

The primary study outcome will assess the temporal trend in levels of Lp(a) in patients with type I acute myocardial infarction. We will compare the change (absolute and percent) in Lp(a) from the acute myocardial infarction event to subsequent measures using paired t-tests within each population group (non-African and African ancestry). We will also compare the between individual time points, using paired t-tests within each population group. Mixed effects and generalized estimating equation (GEE) models will be used to compare the time courses for Lp(a) change using all available time points within each population group. For all analyses, p-values <0.05 will be used to indicate statistical significance.

Sample Size Considerations

We are providing power calculations for the primary endpoint:

Power calculations for this study are based on the primary outcome of change in Lp(a) levels between the acute myocardial infarction setting and that obtained during a subsequent, stable setting. We anticipate non-acute myocardial infarction median (interquartile range) Lp(a) levels of 17 (7-47) mg/dL (33 nmol/L (12-98) as is true in the general adult US population.[45] The standard deviations for the acute phase (Day 0 and Day 30) as well as for the 30-day follow-up are derived from the ongoing EVACS study.[46] As shown in **Table 4** the total sample size of 100 participants**)**, using a two-sided p-value of 0.05, will have 80% power to detect a minimum difference of 42 nmol/L in Lp(a) at Day 30 (12.5% difference from baseline; **Table 5**). Based on the differences from general adult US population median of 33 nmol/L to the expected 30 day level post infarction of approximately 45 nmol/L based on what we observed in our EVACS study, we will have appropriate power to detect clinically important differences in Lp(a) levels with our sample size of 100.

Table 4:

N=100	Minimum detectable difference [Lp(a), nmol/L]
Day 0 (SD=101)	
80% Power	29
85% Power	31
90% Power	33
Hospital Day 1-7 (SD=121)	
80% Power	34
85% Power	37
90% Power	40
Day 30 (SD=147)	
80% Power	42
85% Power	45
90% Power	50

Table 5:

N=100	Minimum detectable %-difference from baseline
Hospital Day 1-7 (SD 29)	
80% Power	8
85% Power	9
90% Power	9.5
Day 30 (SD=44)	
80% Power	12.5
85% Power	13.5

90% Power	14.5

In terms of the African American cohort we anticipate that the Lp(a) changes will be larger [47] and based on our experience in the EVACS study, we anticipate approximately 25% of the enrolled subjects will be African American.[46] Furthermore, we anticipate the non-acute myocardial infarction median (interquartile range) Lp(a) levels of 77 (20-200) nmol/L as published from the Dallas Heart Study [47]. As shown in **Table 5** the sample size of approximately 25 African American participants, using a two-sided p-value of 0.05, will have 80% power to detect a minimum difference of 85 nmol/L in Lp(a) at Day 30 (26% difference from baseline; **Table 6**). Based on the differences from the general US adult African American median of 77 nmol/L to expected 30 day level post infarction of approximately 152 nmol/L based on what we observed in our EVACS study, we will have appropriate power to detect clinically important differences in Lp(a) levels with our sample size of 25.

Table 5:

N=25	Minimum detectable difference [Lp(a), nmol/L]
Baseline (SD=101)	
80% Power	60
85% Power	65
90% Power	70
Hospital Day 1-7 (SD=121)	
80% Power	70
85% Power	75
90% Power	85
Day 30 (SD=147)	
80% Power	85
85% Power	90
90% Power	100

Table 6:

N=25	Minimum detectable %-difference from baseline
Hospital Day 1-7 (SD 29)	
80% Power	17
85% Power	18
90% Power	20
Day 30 (SD=44)	
80% Power	26
85% Power	28
90% Power	30

11. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

Study drug:

Evolocumab is supplied as a sterile, single-use, preservative free solution for subcutaneous injection in a disposable, spring-based prefilled autoinjector/pen (AI/pen). The AI/pen contains a 1.0 mL deliverable volume of 140 mg/mL evolocumab or placebo and three 140mg/ml AI/pens will be dispensed by the Johns Hopkins Investigational Drug Service and will be administered by one of the investigators to each study participant. Evolocumab is well tolerated with few side effects. A prior clinical trial reported no significant differences in adverse events (AEs) and serious adverse events (SAEs) between those participants receiving evolocumab and those receiving placebo in a randomized, placebo- controlled trial over three months of administration (Rutherford 2) [48]. Other trials reported no SAEs and no differences in AEs between treatment with the PCSK9 inhibitor and placebo [49]. The most common reasons for withdrawal were unrelated to the study drug. The GAUSS-3 randomized trial comparing evolocumab and ezetimibe reported no difference in AEs between the study arms and no SAEs reported over 24 weeks [50]. A recent large metanalysis of PCSK9 inhibitor trials showed no difference in treatment-emergent AEs or SAEs in evolocumab-treated and placebo-administered patients[51]. Overall, injection site reactions are manageable and rarely lead to discontinuation of the PCSK9 antibody. Other side effects are all comparable to those reported in the placebo groups. Importantly, very few musculoskeletal side effects are reported in major clinical trials and are comparable to those seen in placebo-treated patients, and there is no difference in laboratory abnormalities reflective of liver or kidney damage over 12 weeks of treatment in the evolocumab vs. placebo groups. [51]

Table 4: The most common adverse effects of evolocumab are listed below and do not differ from those which occurred in patients who received placebo. [51]

Most Common Safety Endpoints	Evolocumab (12 week)	Placebo (12 week)	P value
	Pooled event rate (95% CI)		
Musculoskeletal disorders	9.8 (4.1-15.4)	7.1 (1.6-12.6)	0.74
Nasopharyngitis	6.2 (3.6-8.8)	4.2 (2.1-6.3)	0.11
Gastrointestinal disorders	5.6 (2.7-8.4)	5.3 (1.9-8.7)	0.73
Upper respiratory tract infection	4.2 (2.5-5.9)	2.9 (0.3-5.6)	0.96
Headache	3.4 (2.2-4.6)	2.6 (1.5-3.7)	0.33
Injection site reactions	2.2 (1.3-3.1)	1.7 (0.9-2.5)	0.82

PET studies and serum samples:

Intravenous line placement during PET study and venipuncture to obtain serum samples: can cause some slight discomfort when the needle is inserted into the arm vein. The potential side effects of taking blood samples may include dizziness, soreness and/or bruising of the skin for several days. In very rare circumstances, bleeding or infection can develop at the needle puncture site. However, because the procedure is performed by trained personnel using sterile and standard medical practices, that is very unlikely.

Isotope (F-18 fluorodeoxyglucose (FDG) Injections (PET-scans):

The total radiation exposure that a study participant will receive from participating in this research study is 3.1 rem. This is more than the 0.3 rem that the average person in the United States gets each year from natural sources like the sun, outer space, air, food and soil. It is less than the 5 rems of radiation that is allowed each year for people who are exposed to radiation in their jobs.

Cardiac MRI:
The risks of this study are the same as the risks of a patient receiving a clinical cardiac MRI exam with gadolinium. .

All MRI equipment used in this study is the same as the equipment that is used for standard clinical care. There are no studies showing any health hazard associated with magnetic field exposure (Yamaguchi 2011 and Schenck 2000) itself. .

Ferromagnetic objects may experience forces near the MRI scanner and elongated metallic implants may lead to unintended heating around the implant during MRI scanning. The potential for injury related to such objects is well described (Schenck 2000) and patients with such risks are excluded from participating in this part of the study.

Gadolinium chelates used as intravenous contrast media in MRI are considered safe and do not exhibit the nephrotoxicity associated with iodinated media. The injection of contrast intravenously is not painful, but may cause discomfort, tingling or warmth in the lips, metallic taste in the mouth, tingling in the arm, nausea, or headache. These symptoms occur in less than 1% of people and are short-lived. Allergic reactions to contrast are extremely rare and the risk of a severe reaction is less than 1 in 300,000. Recently, gadolinium-based contrast has been reported to cause Nephrogenic Systemic Fibrosis (NSF) in patients on dialysis or renal failure. The FDA has issued warnings with regards to this issue ((FDA), 2010). Those with creatinine clearance of <45 ml/min/m^2 are excluded from participating in this part of the study. .

b. Steps taken to minimize the risks.

To protect against and minimize the potential risks of evolocumab, study participants will undergo a careful history as well as physical examinations at baseline to identify any condition which might increase their risk from the medication but not known to the patients or documented in their medical records. The subcutaneous injections of the study drug (evolocumab or placebo) will be administered by one of the physician investigators. In addition, patients will be evaluated closely at regular intervals following the injection for side-effects. Patients who experience any site injection adverse events will be evaluated.

Trained personnel will perform the blood collection procedure and will make every effort to minimize any risks or discomfort.

MRI Scanning performed using the same MRI Device Safety Protocol used for the device MRI study (Protocol NA_00051707).
Gadolinium-based MR contrast agents are routinely used in clinical practice, and allergic reactions are infrequently reported. Nineteen adverse events occurred in 28,340 subjects (0.07%) while the rate of serious allergic reactions was much less than 0.01% (Am J Roentgenol 2001;176:1385-1388 and Acad Radiol 1999;6:656-664). We have used the suggested language for clinically performed contrast MRI studies in the consent form and added the possible risk of hypotension. The FDA recently issued an advisory on gadolinium contrast agents used in MRI causing Nephrogenic Systemic Fibrosis or Nephrogenic Fibrosing Dermopathy (NSF/NFD) which apparently can occur in patients with severe renal disease and have given a black box warning regarding their use in patients with GFR<30ml/min. To date, the FDA reports that they have had no reports of NSF in subjects with normal or only mildly impaired renal function. Over 90% of cases have occurred with Omniscan. Below is our contrast sliding scale for MRIs.

Contrast:

1. 0.2 mmol/kg (double dose) Gadavist if GFR>=60 ml/min/1.73m2
2. 0.1 mmol/kg (single dose) Multihance if GFR between 45-60 ml/min/1.72 m2
3. No contrast if GFR<45 ml/min/1.73 m2

Medical personnel are available to administer necessary medical care in the event of allergic reactions.

c. Plan for reporting unanticipated problems or study deviations.

All clinically significant unanticipated problems or study deviations will be reported to the DSMB and the IRB according to The Johns Hopkins Medicine Institutional Review Boards published guidelines. The DSMB will include a cardiologist familiar with the management of patients with acute coronary symptoms, a statistician, and an individual with a background in medical ethics. The DSMB will have access to study drug assignment and will make recommendations to Dr. Leucker regarding any safety concerns and continuation of the study.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

Once the data are acquired, they will be assigned a code for each subject with identification secured and accessible only to the principal and other investigators, and the research coordinator. Patient clinical information such as copies of clinical studies and imaging reports will be filed in a locked filing cabinet with access under control of Dr. Leucker, the other investigators, and study coordinators. Patient information summarized and/or converted into electronic form (tables, images, etc.) will be identified by the assigned code. We will abide by the Johns Hopkins Institutional policy that all identifiers in images and data acquired under IRB-approved research protocols must be removed if they leave the institution, for example in presentations.

e. Financial risks to the participants.

All study related costs will be paid from research sources, as stated in the consent form.

12. Benefits

a. Description of the probable benefits for the participant and for society.

It is not known whether the enrolled participants will receive any benefits from the study drug. In general, those participating in clinical trials may benefit by receiving closer follow-up and improved access to physicians (the physician investigators) than do those not participating in such studies. If the results regarding the safety and efficacy outcomes demonstrate a favorable effect of evolocumab, their participation would be expected to benefit future NSTEMI and STEMI patients.

13. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

For each outpatient study visit study participants will receive $75. Additionally, study participants will be reimbursed for transportation and parking expenses and given a $10 meal voucher following procedures requiring prior fasting.

14. Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

There is no cost to the participants related to the study procedures or drugs.

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