Effects of Evolocumab on Platelet Reactivity in Patients with Diabetes Mellitus after Elective Percutaneous Coronary Intervention:

The HI-REACT-SIRIO study

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Drug Name: Evolocumab

Sponsor: Inova Heart and Vascular Institute at Inova Fairfax Hospital, Falls Church, VA

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

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PROTOCOL SYNOPSIS

Title: Effects of Evolocumab on platelet reactivity in patients with Diabetes Mellitus after Elective Percutaneous Coronary Intervention: The HI-REACT-SIRIO study

Short Title: HI-REACT

Rationale: Diabetes mellitus is associated with accelerated atherothrombosis; a 2-to 4-fold greater CV risk of coronary artery disease is observed in subjects with DM in comparison to non-DM patients translating into a higher risk of recurrent cardiovascular complications despite optimal statin and antiplatelet treatment[1-3]. A prothrombotic state associated with increased platelet reactivity is well demonstrated in DM individuals, placing them at higher risk for atherothrombotic events[4]**.** High platelet reactivity determined in type 2 DM patients with coronary artery disease while on chronic dual antiplatelet therapy is associated with a prominently higher risk of adverse cardiovascular events despite optimal statin and antithrombotic therapy[4]; there are therefore unmet needs for drug regimens that can reduce platelet activation in these high-risk patients. Recently available evidence corroborates the role of PCSK9 in influencing platelet reactivity that in turn may impact patient's risk. A relationship among dyslipidemia, high oxLDL levels, elevated plasma fibrinogen levels, and platelet activation has been demonstrated in patients with cardiovascular disease[5,6]. Oxidized LDL can activate platelets via several mechanisms that include interaction with scavenger receptor A and CD36[7,8]. The relation between oxLDL, platelet reactivity and thrombotic risk may be more pronounced in patients with DM and treatment with PCSK9 antibodies may be associated with a significant reduction in platelet reactivity.

Objectives

Primary Objective: To compare the effects of the clopidogrel plus evolocumab therapy versus clopidogrel plus placebo therapy on platelet reactivity measured by adenosine diphosphate (ADP)-stimulated p-selectin by flow cytometry at 30 day post- dose in patients with atherosclerotic vascular disease (ASCVD) on optimal statin therapy as per physician and diabetes mellitus (DM) undergoing elective percutaneous coronary intervention (ePCI) and clopidogrel administration.

Secondary Objectives: To compare the effects of clopidogrel plus evolocumab therapy versus clopidogrel plus placebo therapy on platelet activation and aggregation, oxidation, inflammation, and lipids by determining the following:

1) Unstimulated and ADP-stimulated (except 30 day times point, primary endpoint) p-selectin and GPIIb/IIIa receptor expression and unstimulated CD36, and LOX-1 by flow cytometry.
2) ADP-, collagen-, and TRAP-induced platelet aggregation by light transmittance aggregometry.
3) Plasma hs-CRP, atherox, oxLDL, p-selectin, hs-troponin, and fibrinogen.
4) Lipid profile including LDL-C.
5) Platelet reactivity measured by VerifyNow P2Y12 assay and the prevalence of high on-clopidogrel platelet reactivity (HPR) (>208 PRU).

Study Type: Pospective, single center, double-blind, randomised pharmacodynamic experimental study.

Study Design: This is a randomized study that will be conducted at Inova Heart and Vascular Institute at Fairfax in 150 subjects with ASCVD on optimal statin therapy as per physician and DM undergoing elective PCI. Eligible patients will be randomized for 30 day treatment to either 1) evolocumab 420 mg ; or 2) placebo.

The randomized treatment will be administered in subcutaneous injections at the investigational site. The laboratory assessments will be performed before (baseline), 16-24 hours and 30±5days after randomization.

Study Methodology: This is a double-blind randomized clinical trial of evolocumab versus placebo in patients with ASCVD and DM on clopidogrel and aspirin undergoing ePCI. The study is aimed to assess
1) the effect of evolocumab therapy on platelet activation and reactivity;
2) the effect of evolocumab on biomarkers of platelet activation and inflammation.

The randomization process will start before PCI.

Eligible patients will be randomized equally to either:
1) 420 mg evolocumab; or
2) placebo.
The randomized treatment will be administered in subcutaneous injections.

Patients: This investigation will be conducted in subjects >18 years of age with ASCVD on optimal statin therapy as per physician and DM undergoing ePCI and treated with clopidogrel and aspirin.

Statistical Methodology: The enrollment of 150 patients (75 per group) would give the study a statistical power of 85% (two-sided alpha=0.05) to detect a significant difference in platelet reactivity between the treatment group and placebo group. Categorical variables will be compared using χ^2 test or the Fisher exact test whereas continuous variables will be assessed with independent-samples t-test or the Mann-Whitney U test in case of non parametric distribution of data; normality of data will be checked with the Kolmogorov Smirnov test. Analyses will be performed with SPSS software (SPSS, Inc., Chicago, IL) and $p<0.05$ will be considered significant.

1. INTRODUCTION

1.1 Specific Aims

To measure platelet activation and aggregation, oxidation, inflammation, and lipids at baseline (before randomization), and 16-24 hours and 30-day after randomization by determining the following:

- P**latelet activation markers**:

  Unstimulated and ADP-stimulated P-selectin, activated GPIIb/IIIa, CD36, LOX-1, and oxLDL expressions on platelets by flow cytometry.

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

- **Lipid profile and soluble markers**:
  Lipid profile including LDL-C by conventional laboratory methods
  Soluble markers (oxLDL, hsCRP, p-selectin, atherox and fibrinogen) in plasma by enzyme linked immunoassay.

- Prevalence of high on-clopidogrel platelet reactivity by the VerifyNow P2Y12 assay.

1.2 Hypothesis

Treatment with evolocumab will be associated with an increased reduction in platelet activation and aggregation and biomarkers associated with platelet activation, oxidative stress, and inflammation as compared to placebo.

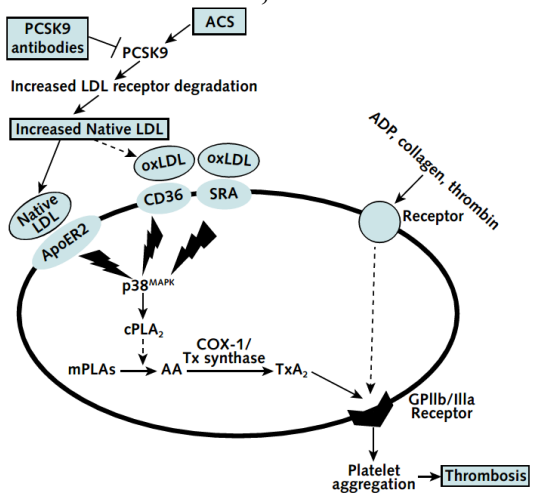
1.3 Background and Significance

Diabetes mellitus (DM) is a major public health problem which currently affects millions of people worldwide. The incidence is expected to double over next 10 years, almost exclusively due to an increase in the prevalence of type-2 DM. Approximately 25 to 30% of all percutaneous coronary interventions (PCIs) are performed in DM patients[1]. The disease is associated with accelerated atherothrombosis; a 2-to 4-fold greater CV risk of coronary artery disease is observed in subjects with DM in comparison to non-DM patients translating into a higher risk of recurrent cardiovascular complications despite optimal statin and antiplatelet treatment[2, 3]. A prothrombotic state associated with increased platelet reactivity is well demonstrated in DM individuals, placing them at higher risk for atherothrombotic events[4]. This status of increased cardiovascular risk persists despite optimal control of traditional risk factors and glycaemia, indicating that the heightened platelet reactivity plays a prominent prognostic role in these patients. Of concern, high platelet reactivity following dual antiplatelet therapy is more frequent in diabetic compared to non-diabetic subjects[9]. This figure stems from the unsolved issue of inadequate responsiveness or 'resistance' to antiplatelet agents, which has a greater prevalence among diabetic patients[10,11]. Numerous sources have demonstrated a direct relationship between low response to clopidogrel or clopidogrel resistance and atherothrombotic events in high-risk DM patients treated with elective PCI and stent implantation[11]. High platelet reactivity determined in type 2 DM patients with coronary artery disease while on chronic dual

antiplatelet therapy is associated with a prominently higher risk of adverse cardiovascular events despite optimal statin and antithrombotic therapy[11]; there are therefore unmet needs for drug regimens that can reduce platelet activation in these high-risk patients.

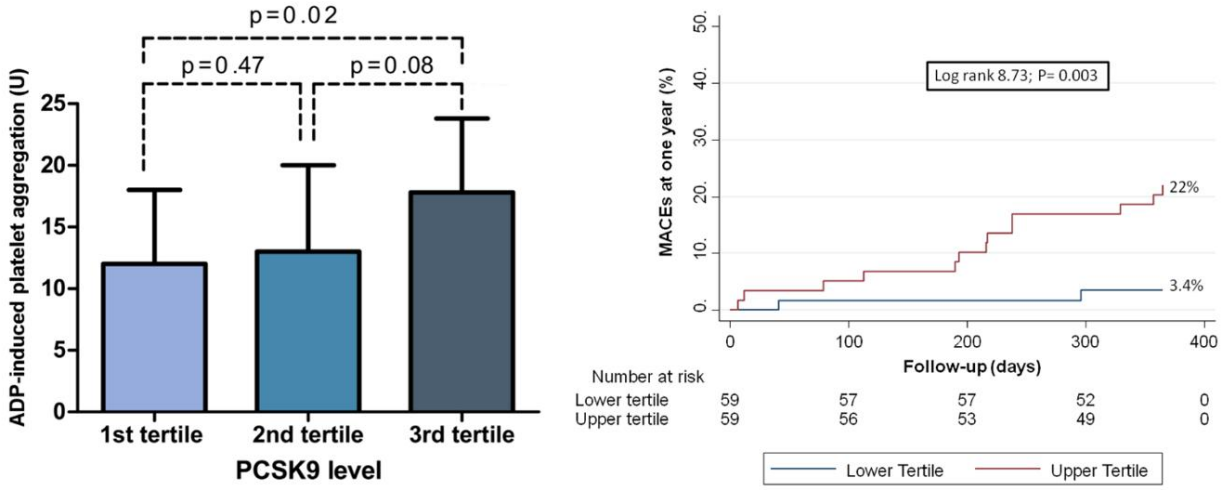
Human monoclonal antibodies against PCSK9 enzyme have been identified as an innovative lipid-lowering strategy. Recently available evidence corroborates the role of PCSK9 in influencing platelet reactivity that in turn may impact patient's risk. A relationship among dyslipidemia, high oxLDL levels, elevated plasma fibrinogen levels, and platelet activation has been demonstrated in patients with cardiovascular disease[5,6]. Oxidized LDL can activate platelets via several mechanisms that include interaction with scavenger receptor A and CD36[7,8]. By enhancing LDL cholesterol clearance from the circulation, PCSK9 antibodies can decrease platelet activation and aggregation induced by native LDL and oxLDL (Figure 2).

Figure 1. Navarese et al. *Ann Intern Med.* 2016;164:600-7.



Recent data already show a stepwise increase of platelet reactivity related to the increased levels of PCSK9 in acute coronary syndrome patients treated with more potent antiplatelet therapies such as ticagrelor and prasugrel (Figure 2). This result is expected to be amplified in elective patients with DM treated with PCI and clopidogrel, that is a less potent antiplatelet agent.

Figure 2. Navarese et al. *Int J Cardiol* 2016;227:644-649



2. STUDY DESIGN AND SUBJECT SELECTION

2.1 Study Type

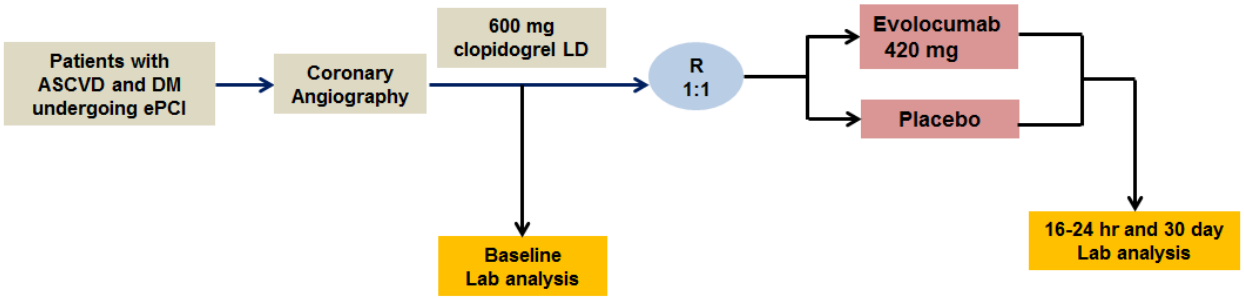
Prospective, single center, double-blind, randomised (1:1) pharmacodynamic study that will be conducted in subjects with ASCVD on optimal statin therapy as per physician and DM undergoing elective PCI. The randomization

process will start before PCI. Eligible patients will be randomized to either

1) evolocumab 420 mg; or
2) placebo.

The randomized treatment will be administered in subcutaneous injections. The laboratory assessments will be performed before (baseline), and 16-24 hours and 30±5days after randomization (see Figure 1).

Figure 1 Design of the HI-REACT-SIRIO study.



Laboratory Analysis

Platelet activation markers:
Unstimulated and ADP-stimulated P-selectin, activated GPIIb/IIIa, CD36, LOX-1, and oxLDL expressions .
Platelet Aggregation:
ADP-, collagen and TRAP-induced platelet aggregation.
Lipid profile and soluble markers:
Lipid profile including LCL-C
Soluble markers (oxLDL, hsCRP, p-selectin, atherox and fibrinogen).
Prevalence of high on-clopidogrel platelet reactivity by the VerifyNow P2Y12 assay.

2.2 Setting

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

2.3 Duration of Study

Subject participation will be 30±5 days from the randomization.

2.4. Number of Subjects

One hundered fifty patients with ASCVD on optimal statin therapy as per physician and DM undergoing ePCI and clopidogrel administration will be randomized to evolocumab or placebo therapy.

2.5. Study Population

2.5.1. Gender of subjects

The study's intended population is inclusive of both genders (males and females).

2.5.2. **Age of Subjects**

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

2.5.3 **Racial and Ethnic Origin**

The study's intended population is inclusive of all racial and ethnic groups and subgroups.

2.5.4 **Vulnerable Population**

Children, pregnant women, institutionalized persons, and persons with decisional incapacity will be not be enrolled in this study. Participants whose primary language is Spanish will have access to translation/interpreter services as provided by the Inova Health System. An IRB approved translated consent form will be provided for the consenting process.

2.6. Recruitment

Recruitment will occur at the Inova Fairfax Hospital. The expected length of the recruitment period is 12 months. If the study conduct (e.g. recruitment rate; drop-out rate; data quality; protocol compliance) does not suggest a proper completion of the trial within the reasonable time frame as agreed upon, the recruitment period may be extended to reach the desired sample size.

2.7 Inclusion criteria: Subjects will qualify for trial participation if ALL criteria below are met:

- Male or female, >18 years of age
- Patients with ASCVD on optimal statin therapy as per physician indication
- Hemodynamically stable patients undergoing elective PCI for coronary artery disease
- Patients with DM will be defined as:

  Patients with Type 1 or type II DM receiving treatment with oral medications or insulin

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

- Patients undergoing emergent PCI for coronary artery disease
- Patients on any PCSK9 inhibition treatment.
- Patients with a history of a serious hypersensitivity reaction to Repatha.
- Patients on any oral thrombin inhibitors
- Patients on factor Xa inhibitors
- Use of cangrelor or Cilostazol, therapy during PCI
- Use of any GPI's during PCI
- Use of any parenteral direct thrombin inhibitors during PCI
- Patients with recent ACS (≤1 month)
- Patients on Clopidogrel therapy with baseline platelet reactivity <43% ADP-induced aggregation as measured by light transmittance aggregometry using 5 μM ADP)
- Patients on dual antiplatelet treatment (DAPT) with ticagrelor or prasugrel
- Patients undergoing urgent/emergent PCI for stent thrombosis
- Participation in any investigational study within the last 60 days.
- Severe renal dysfunction, defined as an eGFR <20 mL/min/1.73 m2 at screening
- Active liver disease or hepatic dysfunction, defined as AST or ALT >3 x ULN as determined by central laboratory analysis at screening

- Recipient of any major organ transplant (e.g., lung, liver, heart, bone marrow, renal)
- Known major active infection or major hematologic, renal, metabolic, gastrointestinal, or endocrine dysfunction in the judgment of the investigator
- Malignancy (except non-melanoma skin cancers, cervical in situ carcinoma, breast ductal carcinoma in situ, or stage 1 prostate carcinoma) within the last 5 years
- Subject has received drugs via a systemic route that have known major interactions with background statin therapy within 1 month before randomization or is likely to require such treatment during the study period (e.g. cyclosporine, clarithromycin, HIV protease inhibitors, gemfibrozil)
- Female subject who is unwilling to use at least 2 effective birth control methods* for at least 1 month before screening and 15 weeks after the end of treatment with investigational products, unless the subject is sterilized or postmenopausal.
- Subject is pregnant or breast feeding, or planning to become pregnant or to breastfeed during receipt of investigational products and within 15 weeks after the end of study treatment
- Known previous hypersensitivity reaction/s to the investigational products' active components and excipients.
- Subject likely to not be available to complete all protocol-required study visits or procedures, to the best of the subject's and investigator's knowledge
- History or evidence of any other clinically significant disorder, condition, or disease other than those outlined above that, in the opinion of the investigator, may compromise the ability of the subject to give written informed consent, would pose a risk to subject safety or interfere with the study evaluation, procedures, or completion.

**Acceptable methods of contraception (birth control) while taking part in this study are:*
• Total Abstinence (no sexual intercourse), absence of menstrual periods in women for more than one year after menopause (change of life), sterilization surgery, including tubal ligation (tubes tied) or hysterectomy (removal of the uterus or womb) in women or a vasectomy in men.
• Oral contraceptives (birth control pills), intrauterine device (IUD), implantable or injectable contraceptives (Norplant or Depo-Provera), contraceptive patch, vaginal ring or use of condom with spermicide. These methods must be used exactly as directed.

3. STUDY METHODS AND PROCEDURES

3.1 Allowed treatments

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

1. Aspirin: 325 mg loading dose followed by 81 mg qd.
2. Clopidogrel: 600 mg loading dose for clopidogrel naïve patients administered in the catheterization laboratorybefore the start of the ePCI or just following the ePCI, followed by 75 mg per day maintenance dose.
3. Unfractionated heparin (UFH): as needed (e.g., 2,000 to 5,000 U) to achieve an ACT of 200 to 250 s.

Subjects who receive other P2Y12 receptor blockers aside from clopidogrel (ticlodipine, cangrelor, ticagrelor, and prasugrel), cilostazol, GP IIb/IIIa inhibitors, direct thrombin inhibitors, factor Xa inhibitors, and warfarin will be excluded from the study.

3.2 Consent

Eligible subjects may only be included in the study after providing written, IRB-approved informed consent, The investigator or qualified designee will explain the study and answer all questions to the prospective subject. If the subject is capable of doing so, he/she should indicate assent by personally signing and dating the written informed consent document or a separate assent form. Informed consent must be obtained before conducting any study-specific procedures (i.e., all of the procedures described in the protocol). In this trial, consent can be obtained upon admission to ER, inpatient setting, pre-procedural or procedural area. The process of obtaining informed consent should be documented in the subject source documents and a copy of the informed consent given to the subject.

3.3 Control group

The control group consists of subjects receiving placebo trteatment.

3.4 Randomization

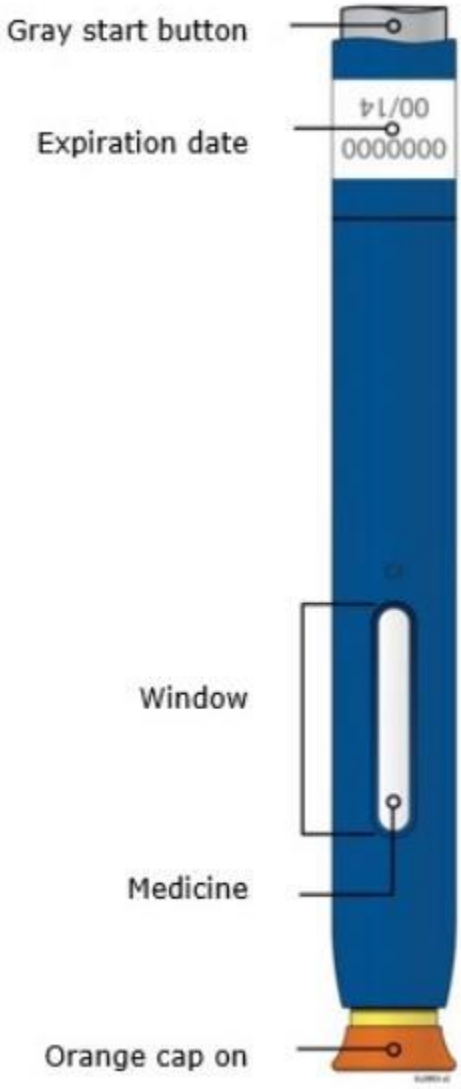
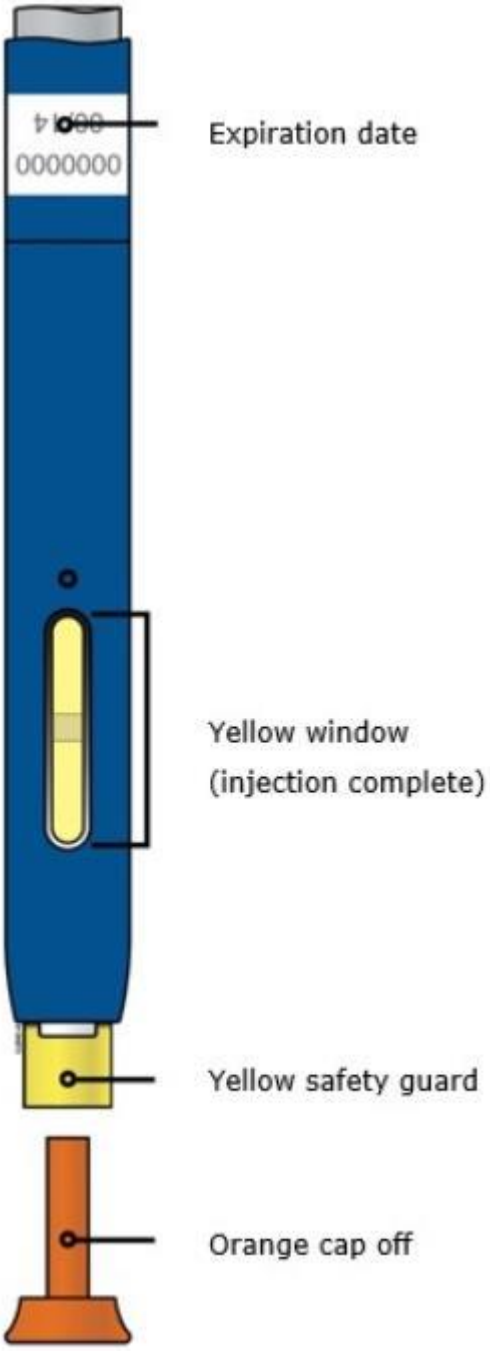
Patients will be randomized to receive 1:1 evolocumab or placebo. Allocation of study treatment will be performed via a web-based interactive randomization system, based on a computer-generated random sequence with a random block size. This study has a double-blind design with evolocumab and matching placebo. The subjects, study site research personnel, academic research center staff, and treating physicians involved in the treatment and/or clinical evaluation of the subjects will not be aware of treatments received.

3.5 Timing of Study Treatment Administration

Qualified subjects will receive the study drug after randomization, within two hours after PCI start time (wire crosses lesion).

3.6 Study Treatment Administration

The study treatments are evolocumab and its matching placebo. The total evolocumab dose to be administered is 420mg. The dosage form and strength of evolocumab supplied for this trial is 140mg/mL solution in a single-useprefilled SureClick autoinjector. To administer 420 mg of evolocumab/placebo, three separate subcutaneous injections, given consecutively within 30 minutes will be administered to randomized subjects. Evolocumab/placebo will be administered by a qualified member of the study staff following successful randomization.

Figure 3: Guide to parts **Before use**	**After use**
	

Important: Needle is inside

Matching placebos will be identical in appearance except that they contain no active substance. Study treatment will be maintained, stored, and distributed by the research pharmacy. Once the study drug is available for administration from the pharmacy, follow the following steps:

Step 1: Prepare
Step 1A: Prepare 3 prefilled autoinjectors for administration. Wait 30 minutes for the autoinjectors to reach room temperature before injecting. Do not heat autoinjectors.
Step 1B; Inspect all SureClick autoinjectors. Check the expiration dates.
Step 1C: Gather all materials needed for the injection and wash hands well with soap and water.
Step 1D: Prepare and clean an injection site. Body areas that can be used include: thigh; abdomen, except for 2-inch area around the navel; and outer area of upper arm. *For the second and third injections, use a different spot than the last injection.* Do not inject into areas where the skin is tender, bruised, red, or hard.
Step 2: Get ready
Step 2A: Pull the orange cap off only when ready to inject. Do not leave the orange cap off for more than five minutes, this can dry out the study drug.
Step 2B: Stretch or pinch the injection site to create a firm surface.
Step 3: Inject
Step 3A: Hold the stretched or pinched skin. With the orange cap off, place the yellow end of the autoinjector on the skin at 90 degrees. Do not touch the gray start button yet.
Step 3B: Firmly push down the autoinjector onto the skin until it stops moving. *You must push all the way down but do not touch the gray start button until ready to inject.*
Step 3C: When ready to inject, press the gray start button. You will hear a click.
Step 3D: Keep pushing the autoinjector down on the skin. Then lift thumb while still holding the autoinjector on skin. Each injection could take about 15 seconds. The medicine window will turn from clear to yellow when the injection is done. You may hear a second click.
Step 4: Finish
Step 4A: When the injection is done, throw away the used autoinjector and orange needle cap.
Step 4B: Check the injection site. If there is blood, press a cotton ball or gauze pad on the injection site. Apply adhesive bandage if needed. *Do not rub the injection site.*

Repeat steps 1B to 4B for the second and third injections. Total dose administered should be 420 mg. Use a different injection site for each injection. Administer injections consecutively within 30 minutes.

3.7 Study Treatment Storage

The prefilled autoinjectors are stored in the original carton and are kept in refrigerated storage. Allowed temperature excursions while in storage is 36°F to 46°F (2°C to 8°C). Once removed from the refrigerator, the autoinjectors should be kept at room temperature at 68°F to 77°F (20°C to 25°C) in the original carton and must be used within 30 days. The prefilled autoinjectors cannot be frozen. Do not use a prefilled autoinjector that has been frozen.

3.8 Endpoints/Outcomes Measurements

3.8.1 Primary endpoints

Absolute difference in ADP-stimulated P-selectin expression 30 days post-dose in between patients treated with clopidogrel plus evolocumab therapy and clopidogrel plus placebo therapy for 30±5days. First absolute difference in p-selectin expression between baseline and 30±5 days post-dose will be calculated in each group followed by estimation of absolute difference between the two groups.

3.8.2 Secondary endpoints

- Absolute differences in unstimulated and ADP-stimulated (except 30 day times point, primary endpoint) p-selectin and GPIIb/IIIa receptor expression and unstimulated CD36, and LOX-1 measured at baseline and 16-24 hours and 30±5day post-randomization in patients treated with clopidogrel plus evolocumab therapy and clopidogrel plus placebo therapy.
- Absolute differences in lipid profile including LDL-C, oxLDL, fibrinogen, hsCRP, p-selectin, atherox (soluble markers) measured at baseline and 16-24 hours and 30±5day post-randomization in patients treated with clopidogrel plus evolocumab therapy and clopidogrel plus placebo therapy.
- Prevalence of high on-clopidogrel platelet reactivity by the VerifyNow P2Y12 assay measured at baseline, and 16-24 hours and 30±5day post randomization.

3.8.3 Clinical endpoints – None

3.8.4 Exploratory analysis

An exploratory analysis of the effect of evolocumab on platelet reactivity in the following subset of patients will be addressed:
i) patients ≥65 years,
ii) previous myocardial infarction (>one year),
iii) glomerular filtration rate (GFR) < 60/min/1.73 m2,
iv) complex coronary artery disease (left main and/or bifurcation and/or multivessel disease).

3.8.5 Blood sampling

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

- P**latelet activation markers**:

  Unstimulated and ADP-stimulated P-selectin, activated GPIIb/IIIa, CD36, LOX-1, and oxLDL on platelets by flow cytometry.

- **Platelet Aggregation**:
  Adenosine diphosphate (ADP)-, collagen and TRAP-induced platelet aggregation by light transmittance aggregometry.

- **Lipid profile and soluble markers**:
  Lipid profile including LDL-C by conventional laboratory methods
  Soluble markers such as oxLDL, hsCRP, p-selectin, Atherox in plasma by enzyme linked immunoassay.

- Prevalence of high on-clopidogrel platelet reactivity by the VerifyNow P2Y12 assay.

4. STATISTICAL CONSIDERATIONS/DATA ANALYSIS

4.1 Sample Size Calculation

Sample Size Calculation: In our previous study in patients loaded with clopidogrel for elective PCI, baseline stimulated P-selectin expression was 45±16% and 23±10% inhibition of P-selectin expression was occurred at 30 days. The absolute change in percent positivity was ~22%[12]. We hypothesize that evolocumab therapy will result in a further 6% absolute reduction in platelet activation (~ 14% percent change from baseline). To detect a 6 percent in absolute reduction of platelet aggregation with evolocumab, assuming a standard deviation of 11, α of 0.05, two-tailed, 85% power, a final sample size of approximately 122 patients (61per group) will be required. To account for drop outs as well as incomplete data assessment at all time points, a final sample size of 150 patients (75 per group) will be recruited.

4.2 Method of Data Analysis

Categorical variables will be compared using χ^2 test or the Fisher exact test whereas continuous variables will be assessed with independent-samples t-test or the Mann-Whitney U test in case of non parametric distribution of data; normality of data will be checked with the Kolmogorov Smirnov test. Analyses will be performed with SPSS software (SPSS, Inc., Chicago, IL) and $p<0.05$ will be considered a significant difference between clopidogrel plus evolocumab therapy versus clopidogrel plus placebo therapy.

4 DATA MANAGEMENT

4.1 Data collection

Designated study site staff will record data required by the protocol into the case report forms (CRFs) and enter it into the electronic database. Authorized research staff will review the CRFs for completeness and accuracy and make any necessary corrections to the data entered into the electronic database.

4.2 Confidentiality, data storage, and retention

Information about study subjects will be kept confidential and managed according to the

requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Each subject screened and enrolled will be assigned a subject identification number (ID) and a list of subjects with their corresponding subject ID will be maintained separately from collected data.

Physical CRFs will be stored in the research site in a locked office and electronic subject data will be locked in a password protected file on a secure internet server, accessed only by authorized research staff. All data records will be stored on site until 2 years after the investigation is formally discontinued. Paper records will be shredded and recycled.

5 VISIT SCHEDULE AND ASSESSMENTS

Informed consent will be obtained from subjects meeting the inclusion criteria and none of the exclusion criteria before the initiation of any study-specific procedures. Subjects will receive 1 dose of the study drug after randomization at the investigational unit. Data analysis is summed up by timepoints: Baseline/pre-PCI, and 16-24 hours, and 30±5 days post-randomization. Required assessments for each study visit are listed in Table 1. Subjects should be seen for all visits on the designated day or according to the allowed window period (see Table 1).

Table 1

Schedule of Events

	V1	V2	V2	EOS
	Screening/ Baseline	**Randomization**	**16-24 Hours Post-First Dose of Evolocumab/ Placebo[1]**	**30 Days (+/- 5days) Post Dose of Evolocumab/ Placebo**
Informed Consent	X			
Inclusion/Exclusion Criteria	X	X		
Medical History	X			X
Review Prior/Concomitant Medications	X	X	X	X
Safety labs (CMP and CK)	X[2]			X
Urine Pregnancy Test[3]	X			X
Physical Examination[4]	X			
Vital signs (BP and HR)	X	X	X	X
Laboratory Measurements	X		X	X
Administer Study Drug (Evolocumab or Placebo)		X		
Adverse Events		X	X	X

[1]Discharge day may coincide with the 16-24 hours post- dose of study drug time point.

[2] CMP laboratory results within 3 months of visit 1 are acceptable. If not available, a CMP needs to be obtained and results reviewed during screening, prior to randomization.

[3]Urine specimen for pregnancy test will be collected on women of childbearing potential at screening and EOS visit.

[4] May be conducted as a nursing assessment if a physical was performed as standard of care ≤1 month prior to screening if no AE/SAE's have occured.

5.1 Timing of Assessments

5.1.1 Baseline/Pre-study drug administration

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

- Obtain written informed consent
- Complete inclusion/exclusion criteria
- Obtain demographic information (i.e. date of birth, gender, and race)
- Record medical history (including medical, surgical, and smoking history)
- Record concomitant medications
- Obtain height and weight
- Obtain and record pre-PCI vital signs
- Perform complete physical examination to evaluate the general status of the subject and to further elucidate patient symptoms, risk factors, or concerns that may increase the subject's risk for adverse reactions to the study treatment
- Obtain safety laboratory results (comprehensive metabolic panel) to check for renal and hepatic function (see exclusion criteria)
- For women of childbearing potential, a urine pregnancy test will be performed
- Obtain blood specimen for platelet activation, aggregation, and biomarkers prior to ePCI. Laboratory specimen should be obtained prior to clopidogrel loading dose
- Adverse events collection once informed consent obtained

5.1.2 Randomization

When a subject qualifies for enrollment/randomization, the clinical site staff will access the web-based interactive randomization system and allocate the patients for particular treatment. The randomization system is based on a computer-generated random sequence with a random block size. The subject will be randomized to receive either 1) 420 mg evolocumab or 2) placebo.

5.1.3 Administration of study drug

The subject will receive the study drug after randomization, once PCI commences. PCI will be perfomed as according to institutional guidelines and standards of care including parenteral anticoagulant and oral anti-platelet if indicated. The 420 mg of evolocumab or placebo will be administered, subcutaneously, within two hours after PCI start time (wire crosses lesion)(see section 3 for study treatment administration).

If a randomized subject received any of the exclusionary medications specified in section 2.8 or does not undergo PCI, the subject will not receive the study drug and will be exited

from the study. The reason for non-intervention must be documented in the subject's medical records.

5.1.4 16-24 hours post-study drug administration

Subjects are expected to be seen as an inpatient during this timepoint or may coincide with the subject's discharge. During this time point, the following study procedures will be completed:

- Obtain laboratory specimens for platelet activation, aggregation, and biomarkers.
- Record any changes in the subject's medical condition, concomitant medications.
- Review and record adverse events.
- Assess for continued subject eligibility.
  Provide patient education about study drug (see section 5.3).
- Provide subject with wallet medication card.
- Set up an appointment for an outpatient visit at the investigational site 30 days (+/-5 days) post- dose of study drug.

5.1.5 End of study visit/ 30±5 days post-dose of study drug

Subjects will return as an outpatient to the research site 30 days (+/- 5 days) post- dose of evolocumab/matching placebo to obtain laboratory measurements for platelet activation, aggregation, and biomarkers, and to review and record changes in concomitant medications, safety lab, pregnancy and adverse events.

5.2 Safety Assessments

Safety assessments will consist of monitoring and recording of adverse events and serious adverse events (see section 6). The occurrence of adverse events should be sought by non-directive questioning of the subject at each visit during the study. AEs may also be detected when these are volunteered by the subject during or between visits or through physical examination, laboratory test, or other assessments. Medical conditions/diseases present before starting the study drug are considered AEs only if they worsen after starting the study drug. Abnormal laboratory values or test results constitute AEs only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy. Abnormal values that constitute a SAE or lead to discontinuation of administration of study drug must be reported and recorded as an AE. Adverse event collection will commence when informed consent is obtained.

Additional assessments required to ensure safety of subjects should be administered as deemed necessary by the investigator on a case by case basis.

Patients experiencing adverse events should be followed clinically until their health has returned to baseline status or until all parameters have returned to normal or have otherwise been explained. It is expected that the investigator will provide or arrange appropriate care for the patient if necessary. The principal investigators are responsible for ensuring that all staff involved in the study are familiar with the content of this section. This information should be captured in the source document at each visit.

5.3 Patient Education

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

5.4 Study Drug Administration

Only trained research personnel will administer study drug to qualified subjects. The study drug will be administered via subcutaneous injection using outer area of upper arm, thigh or stomach (abdomen) area (except for two inch area around the belly button. Study drugs will be stored and administered as according to manufacturer's instructions.

6 ADVERSE EVENTS

6.1 Adverse Event (AE) Definitions

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

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

An adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event. An adverse event or suspected adverse reaction is considered "unexpected" if it is not specifically mentioned as occurring with the particular drug under investigation. Information about common side effects already known about the study drug/s drug can be found in the study drug/s package inserts. This information will be included in the subject's informed consent and should be discussed with the subject during the study as needed.

6.2 Serious Adverse Event (SAE) Definition

An SAE is defined as an event that:

- is fatal or life-threatening;
- results in persistent or significant disability/incapacity;
- constitutes a congenital anomaly/birth defect;

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

6.3 AE Grading Scale

The descriptions and grading scales found in NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be used for AE reporting. Each AE term is associated with a 5-point severity scale.

6.4 AE Collection and Reporting

All SAEs and AEs which are not serious but which lead to permanent discontinuation of study medication will be captured in the CRF. Non-serious AEs which do not lead to discontinuation of study medication will not be collected. AE collection will commence once informed consent is obtained. A detected SAE or an AE deemed related to study drug that led to permanent study drug discontinuation should be followed until its resolution or until the subject completes the study.

6.5 Procedures for Recording and Reporting of Adverse Events

All AEs, as specified in section 6.4, will be reported to the principal investigator. For both serious and non-serious AEs, the investigator has the primary responsibility for AE. identification, documentation, grading, and assignment of attribution to the study treatment/intervention. The sponsor will consider the investigator's view when assessing the safety of the drug and determining whether to report expeditiously to the FDA, local institutional review board (IRB), and other regulatory agencies.

AEs, as specified in section 6.4, occurring once informed consent is obtained through the last day of study participation must be recorded on the AE CRF with the following information:

- The intensity grade (grade 1, 2, 3, 4, 5; see CTCAE v4.03 grading)
- The relationship to the study drug(s)
- Attribution: An assessment of the relationship between the AE and the medical intervention (i.e., study drug administration). After naming and grading the event, the clinical investigator must assign an attribution to the AE using the following attribution categories:

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

For serious adverse events not previously documented in study drug/s Package Insert (new occurrence) and are thought to be related to the study drug/s, the investigator may urgently require further information from the investigator for Health Authority reporting. Amgen or designee may need to issue an IND Safety Letter (Investigator Notification) to inform all investigators involved in any study with the same drug that this SAE has been reported.

All suspected unexpected serious adverse reactions (SUSARs) related or possibly related to evolocumab and their follow-up reports must be reported to Amgen within 24 hours of submission to the regulatory agency, IRB or IEC. A copy of any safety report involving an Amgen drug (e.g. evolocumab) submitted to the regulatory agency, IRB or IEC, must be faxed to Amgen, within 24 hours of such submission.

6.6 Reporting of Pregnancies

Pregnant, lactating women, or women planning to become pregnant or to breastfeed during receipt of investigational products and within 15 weeks after the end of study treatment are excluded from participating in this trial. Women of child-bearing potential recruited into the study must have a negative urine pregnancy test prior to initiation of evolocumab therapy (screening visit) and will undergo urine pregnancy testing at the end of study visit. Women of child-bearing potential must agree to use at least 2 forms of medically accepted method of contraception during the entire study duration. Male participants will be advised to use a medically accepted method of contraception during the study duration. If a female partner conceives and becomes pregnant while the male subject is participating in this study, the sponsor will be notified as per the procedures described above. The sponsor will report all pregnancies and pregnancies occurring in the partner of a patient participating in the study or potential infant exposure through lactation within 10 calendar days of sponsor's awareness to Amgen.

6.7 Adverse Event Treatment

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

7 SUBJECT COST AND FUNDING

This is an investigator initiated study funded by Amgen Inc. Amgen, Inc will provide the investigational products.

The subject or their insurance company will not be billed for this study. All study related tests and procedures will be paid for by the research site.

8 SUBJECT COMPENSATION

A total of $120 financial compensation will provided for study participation with the following breakdown each visit (Visit 1: $40 Visit 2: $40, and EOS:$40) to cover transportation, parking, and meal expenses. Compensation will only be paid for completed visits. Payment will be received by subjects after completion of each visit using reimbursement card of direct deposit as according to Inova Healthy System policy

9 CONFLICTS OF INTEREST

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

10 FACILITIES AND EQUIPMENT

The research site is equipped with its own laboratory equipment, which includes state of the art technologies for platelet assays, centrifuges, refrigerators, and freezers for study specimen processing and storage. For outpatient visits, subjects will be seen in the site's outpatient clinic room equipped with supplies and equipment for subject assessment.

11 OUTSIDE CONSULTANTS/COLLABORATORS

There are no outside consultants/collaborators participating.

10 CONTRACTURAL AGREEMENTS

There are no outside consultants/collaborators participating.

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