Antiplatelet Effects of Evolocumab in Patients with Peripheral Arterial Disease

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Drug Name: Evolocumab

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

Title: Antiplatelet Effects of Evolocumab in Patients with Peripheral Arterial Disease

Short Title: Antiplatelet effects of Evolocumab

Rationale: Peripheral arterial disease (PAD) affects about 8.5 million Americans aged ≥40 years. PAD is the third leading cause of atherosclerotic vascular morbidity after coronary heart disease and stroke.[1,2] Statin therapy has been shown to improve walking performance, resting and post-exercise ABI and claudication.[3] In addition, higher statin doses and lower LDL-cholesterol levels have been shown to be independently associated with improved outcomes in PAD patients.[4] The latter finding indicates that significantly lower LDL-cholesterol may be associated with better clinical outcomes and supports proprotein convertase subtilisin/kexin type 9 (PCSK9) antibody therapy in patients with PAD. A relation between dyslipidemia, oxLDL levels, high plasma fibrinogen levels and platelet activation has been demonstrated in patients with CVD.[5-7] In addition to LDL cholesterol reduction and plaque stabilization, other mechanisms of PCSK9 antibodies may further mitigate cardiovascular risk in patients with PAD.

Objectives

Primary: To study the effect of evolocumab + high dose statins on platelet activation and platelet aggregation.

Secondary: To study the effect of evolocumab + high dose statins on lipid profile, oxLDL, soluble LOX-1 receptor, fibrinogen, hsCRP, p-selectin, atherox (Soluble markers), Urinary 11-dh-TxB2 and 8-iso-prostaglandin $F2_{\alpha}$ levels.

Exploratory: To study the effect of evolocumab + high dose statins on platelet-fibrin clot characteristics and shear-induced platelet aggregation.

Study Type: Single arm-open label study design.

Study Design: This is a phase IV, prospective pharmacodynamic study.

Study Methodology: This investigation will be conducted in subjects >18 years of age with PAD. Platelet activation and aggregation, and biomarkers associated with platelet activation, oxidative stress, and inflammation will be assessed at the screening (baseline), after 8 weeks of high-dose statins and 24 hours and 8 weeks after high dose statin + evolocumab therapy (study flow diagram).

Patients: Sixty patients with a history of Peripheral Artery Disease (PAD)including carotid artery disease.

Statistical Methodology: Categorical variables will be compared using χ^2 test or the Fisher exact test whereas continuous variables will be assessed using either paired test or Wilcoxon signed rank test after checking for normal distribution. Analyses will be performed with SPSS software (SPSS, Inc., Chicago, IL) and $p<0.05$ will be considered significant

INTRODUCTION

1.1 Specific Aims

To measure platelet activation and aggregation, soluble biomarkers, clot characteristics and shear-induced platelet aggregation at the baseline, after 8 weeks of high-dose statins and 24 hr and 8 weeks after high-dose statins + evolocumab therapy (study flow diagram**).**

- **Platelet Aggregation**:
  Adenosine diphosphate (ADP)-, Arachidomic acid (AA)-, and collagen-induced platelet aggregation by light transmittance aggregometry.
- P**latelet activation markers**:

  P-selectin, CD36, LOX-1, basigin (CD147, receptor for glycoprotein VI), oxLDL on platelets by flow cytometry.
- **Lipid profile and soluble markers**:
  Lipid profile including LDL-C measurement by conventional laboratory methods and soluble markers such as oxLDL, LOX-1 receptor, fibrinogen, hsCRP, p-selectin, Atherox in plasma by enzyme linked immunoassay.
- **Platelet-fibrin clot characteristics:**
  Thrombin-induced platelet-fibrin-clot strength, time to initial thrombin generation, and functional fibrinogen by thrombelastography (TEG6S).
- **Shear-induced platelet aggregation:**
  Shear-induced platelet aggregation by T-TAS perfusion flow chamber analysis.
- **Urine 11-dh-TxB2 and 8-iso-prostaglandin F2$_\alpha$**

  Urinary 11- dehydrothromboxane B2 level using The AspirinWorks™ ELISA (Corgenix) and urinary 8-iso-prostaglandin F2$_\alpha$ analysis using Cayman's 8-Isoprotane ELISA kit.

To measure ankle-brachial index (ABI) at baseline, after 8 weeks of high dose statin therapy (Visit 2), and 8 weeks after high-dose statins + evolocumab therapy (Visit 5) to determine the severity of peripheral arterial disease (PAD) and response to evolocumab treatment on endothelial function.

1.2 Hypothesis

Treatment with evolocumab plus high-dose statin is associated with a reduction in platelet activation and aggregation and biomarkers associated with platelet activation, oxidative stress, and inflammation.

1.3 Background and Significance

Peripheral arterial disease affects about 8.5 million Americans aged ≥40 years. PAD is the third leading cause of atherosclerotic vascular morbidity after coronary heart disease and stroke.[1,2] PAD is a manifestation of diffuse systemic atherosclerosis that coexists with coronary artery and cerebroarterial disease. Patients with PAD often have a very large atherosclerotic burden and large atherosclerotic burden has been associated with heightened platelet reactivity measured in systemic circulation.[8] About 10–20% of people with PAD have intermittent claudication, another 50% have atypical leg symptoms, and those without exertional leg pain have poor mobility compared with individuals without peripheral symptoms. Patients with PAD have roughly a three-fold increase in risk of mortality compared with those without peripheral artery disease.[2] Ankle-brachial index (ABI) measurement is a well-established tool, to identify PAD.

With the expanding size of the elderly population, PAD is similarly a major growing clinical problem with limited current therapeutic options. Patients with PAD primarily die from thrombotic events - heart attack and stroke. The current antithrombotic agents administered for prophylaxis are aspirin and clopidogrel.[9] High LDL is a major risk factor for PAD and therefore lipid-lowering therapy constitutes another important therapeutic intervention for patients with PAD. Statin therapy has been shown to improve walking performance, resting and post-exercise ABI and claudication.[3] In addition, higher statin doses and lower LDL-cholesterol levels have been shown to be independently associated with improved outcomes in PAD patients.[4] The latter finding indicates that significantly lower LDL-cholesterol may be associated with better clinical outcomes and supports proprotein convertase subtilisin/kexin type 9 (PCSK9) antibody therapy in patients with PAD.

Monoclonal antibodies against PCSK9 are innovative agents that provide very potent LDL reduction when administered on top of statins. PCSK9 antibodies prevent LDL receptor degradation and enhance circulatory LDL cholesterol clearance. The lower LDL cholesterol levels potentially enhance the stabilization of vulnerable plaque in both culprit and non-culprit vessels following the index event.[10] Increased PCSK9 levels have been correlated with cardiovascular event occurrence.[11,12] Elevated LDL levels result in the generation of proinflammatory oxidized (ox) LDL and augmentation of plaque vulnerability. Levels of circulating lectin-like oxLDL receptor-1 (LOX-1), a major ox-LDL receptor, are elevated in patients with ACS, with a diagnostic sensitivity and specificity superior to high-sensitivity C-reactive protein to identify patients with ACS. LOX-1 receptor expression is inducible by proinflammatory stimuli in a dynamic fashion.[13,14] LOX-1 receptor activation induces oxLDL uptake, increased production of cell-surface adhesion molecules and a state of oxidative stress and inflammation- key steps involved in the progression of atherosclerosis and destabilization of plaque. Recent studies indicate a cross-reactive mechanism of PCSK9 and LOX-1 that leads to a mutually amplified proinflammatory response.[15]

In addition, a relation between dyslipidemia, oxLDL levels, high plasma fibrinogen levels and platelet activation has been demonstrated in patients with CVD.[5-7] OxLDL activates platelets and promotes adhesion to dysfunctional endothelium.[16,17] A proposed mechanism of oxLDL-induced platelet activation includes binding of oxLDL to the ApoB/E receptor (APOB/E-R) and platelet endothelial adhesion molecule (PECAM-1) leading to the generation of TxA2 mediated by p38MAPK and cytosolic phospholipase A2 (cPLA2). The latter platelet activation is synergistic with platelet responses to other agonists such as

adenosine diphosphate, collagen, and thrombin leading to platelet aggregation. The interaction between oxLDL and scavenger receptor A (SRA) and CD36 are also known to activate platelets via cPLA2 and p38MAPK-mediated mechanisms. A recent animal study suggested that platelet activation associated with oxLDL and hyperlipidemia is due to generation of reactive oxygen species (ROS) through a CD36-protein kinase C (PKC) pathway and modulation of cGMP signaling.[18] Finally, plasma PCSK9 levels were also positively and independently correlated with increased platelet count and plateletcrit.[19]

The mainstay therapy of patients with PAD is aspirin and statin therapy. Chaudhary et al. demonstrated that patients with atherosclerosis on aspirin and lipid lowering therapy was associated with significantly lower levels of inflammation and thrombogenicity as measured by urinary 11-dTxB2.[20] Secondly, another urinary measure which has shown to be a marker of inflammation /oxidative stress is Urinary 8-iso-PGF2α . There is no information on the relationship of PCSK9 therapy on urinary 11-dTxB2 and 8-iso-PGF2α levels. [21]

2 STUDY DESIGN AND SUBJECT SELECTION

This is an single-arm open label study that will be conducted in subjects with PAD including carotid artery disease. Sixty subjects will be treated with high dose statins for 8 weeks followed by 8 weeks of high dose statin + evolocumab (420mg Q 4weeks x 2 doses) therapy. The laboratory assessments (see section 2.2) will be performed at the screening (baseline), after 8 weeks of high-dose statin therapy, and 24 hr and 8 weeks after high dose statin + evolocumab therapy (see Figure 1).

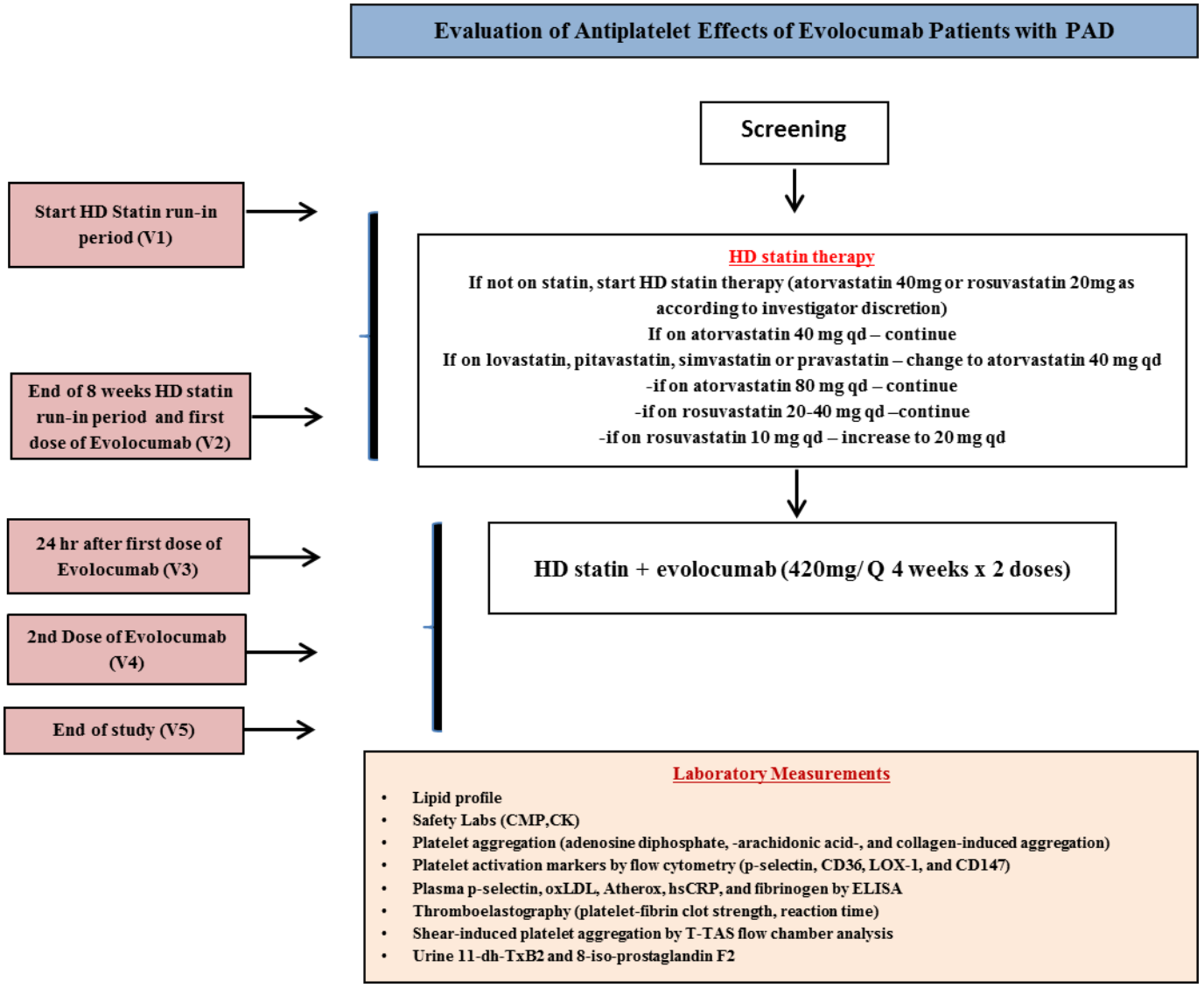


Figure 1.

2.1 Recruitment

Recruitment will occur at the Inova Heart and Vascular Institute. 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.

2.1.1 **Eligibility criteria**

- Inclusion criteria
  - History of Peripheral Artery Disease (PAD).
    Defined as one or more of the following:
    - Documented history of PAD
    - Previous limb or foot amputation for arterial vascular disease (i.e., excludes trauma),
    - Carotid artery disease (defined as >50% stenosis or prior revascularization)
  - Subject may be of either sex and of any race, and must be >18 years of age
  - Subject agrees to not participate in any other investigational or invasive clinical study for a period of 4 months during the study period
  - Subject must be willing and able to give appropriate informed consent
  - The subject is able to read and has signed and dated the informed consent document including authorization permitting release of personal health information approved by the investigator's Institutional Review Board (IRB)
  - Subjects who are either statin-naïve or already on statin and are willing to be started on high dose (HD) statin therapy:
    - If not on statin→start HD statin therapy (atorvastatin 40mg or rosuvastatin 20mg as according to investigator discretion)
    - If on atorvastatin 40 mg once a day→continue
    - If on lovastatin, pitavastatin, simvastatin, or pravastatin→change to atorvastatin 40 mg once a day
    - If on atorvastatin 80mg once a day→continue
    - If on rosuvastatin 20 to 40mg once a day→continue
    - If on rosuvastatin 10mg once a day→increase to 20mg once a day

- **Exclusion criteria:** Subjects will be excluded from entry if ANY of the criteria listed below are met:
  - Prior use of any PCSK9 inhibition treatment
  - Participation in any investigational study within the last 60 days.
  - Severe renal dysfunction, defined as an eGFR <20 mL/min/1.73 m2, or creatinine kinase (CK) level at 10 X ULN 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
- Subjects treated with any anticoagulant
- Patients treated with Prasugrel or Ticagrelor
- 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.

2.2 Study methods

2.2.1 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 (n=3) will be used for flow cytometry, light transmittance aggregometry and thrombelastography. Tube containing hirudin will be used for shear-induced platelet aggregation and tubes containing 3.2% trisodium citrate will be used for platelet activation marker analysis.

- **Platelet Aggregation**
  Platelet aggregation will be assessed in platelet-rich plasma using a Chronolog Lumi-Aggregometer (model 490-4D) with the AggroLink software package after stimulation with 2 and 5µM ADP, 1.2 mM AA and 4ug/ml collagen.

- **Platelet Activation by Flow cytometry**

  Expressions of p-selectin, CD36, LOX-1, basigin (CD147, receptor for glycoprotein VI), and oxLDL on platelets will be assessed by flow cytometry using respective antibodies.

- **Platelet-fibrin clot characteristics by TEG6S**

  The TEG6s instrument is a microfluidic fully automated cartridge-based device.

- **Lipid profile and Soluble Biomarkers**

  After collection, plasma samples will be frozen at -70°C until analysis by commercially available enzyme linked immunoassay kits and ACL TOP 300 coagulation analyzer. Lipid profile will be assessed by conventional laboratory method and soluble markers such as oxLDL, LOX-1 receptor, fibrinogen, hsCRP, p-selectin, Atherox in plasma by enzyme linked immunoassay.

- Safety Labs including Comprehensive Metabolic Panel (CMP) and Creatine Kinase (CK).

2.2.2 **Urine Sampling**

Ten (10) mL of a spot urine specimen will be collected at baseline, 8 weeks post high-dose statin therapy and 8 weeks post first dose of evolocumab.

- **Urine 11-dh-TxB2 and 8-iso-prostaglandin $F2_{\alpha}$**

  Urine 11-dh-TxB2 concentrations by AspirinWorks test kit, an enzyme linked immunoassay (ELISA).
  Urinary 8-iso-$PGF_{2\alpha}$ analysis by 8-iso-Prostaglandin $F2_{\alpha}$ ELISA Kit (Cayman Chemical Co. #516351).

3 STATISTICAL CONSIDERATIONS/DATA ANALYSIS

3.1 Primary and secondary endpoints

Primary endpoint

Relative difference in maximal ADP-induced platelet aggregation between high-dose statin therapy and high-dose statin plus evolocumab therapy
Relative difference = 100 * (baseline on high dose statin value – post high dose statin plus evolocumab value)/ baseline on high dose statin value

3.1.1 **Secondary Endpoints**

- Relative differences in AA- and collagen-induced platelet aggregation between high dose statin therapy and evolocumab plus high-dose stain therapy.

- Relative differences in platelet bound p-selectin, oxLDL, soluble LOX-1 receptor, between high dose statin therapy and evolocumab plus high-dose stain therapy.
- Relative differences in lipid profile, oxLDL, soluble LOX-1 receptor, fibrinogen, hsCRP, p-selectin, atherox (soluble markers) between high dose statin therapy and evolocumab plus high-dose stain therapy.
- Relative differences in platelet-fibrin clot characteristics and shear-induced platelet aggregation between high dose statin therapy and evolocumab and high-dose stain therapy
- Relative differences in ABI between high dose statin therapy. and evolocumab and high-dose stain therapy
- Relative differences in urinary 11-dh-thromboxane B2 levels between high dose statin therapy and evolocumab plus high-dose statin therapy.
- Relative differneces in urinary 8-iso-Prostaglandin $F2_{\alpha}$ levels between high dose statin therapy and evolocumab plus high-dose statin therapy.

3.2 Statistical calculations

Categorical variables will be compared using χ^2 test or the Fisher exact test whereas continuous variables will be assessed using either paired test or Wilcoxon signed rank test after checking for normal distribution. Analyses will be performed with SPSS software (SPSS, Inc., Chicago, IL) and $p<0.05$ will be considered a significant difference between high-dose statin therapy and evolocumab plus high-dose statin therapy.

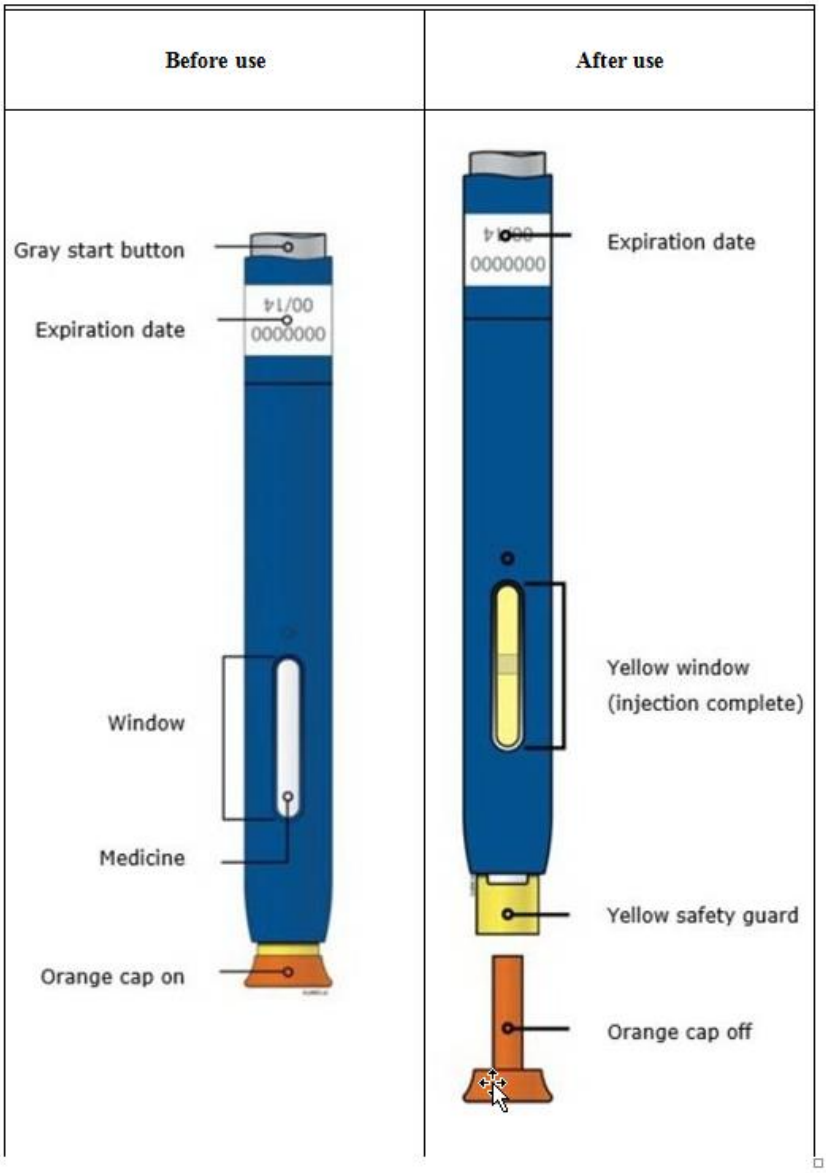
No prior data is available regarding direct antiplatelet effects of evolocumab. Sample size calculation is mainly based on our previous experience in pharmacodynamic studies. In this study, 50 patients are needed to detect the absolute difference of 14% (~20% relative difference) in ADP-induced aggregation after evolocumab plus high dose statins compared to previous high dose statin therapy alone in these same patients with a power of 91%, a two sided alpha = 0.05 and SD of 30%. Assuming a 16% drop out rate, we need 60 patients in total. Calculations based on a paired t-test.

3.3 Clinical endpoints – None

4.0 EVOLOCUMAB

Two doses of evolocumab will be administered in this trial, given 4 weeks apart (see visit schedule). Each 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-use prefilled SureClick autoinjector. To administer 420 mg of evolocumab, three separate subcutaneous injections, given consecutively within 30 minutes will be administered to subjects. Evolocumab will be administered by a qualified member of the study staff.

Figure 3 Guide to Parts



4.1 Study Treatment Administration
Study treatment will be maintained, stored, and distributed by the research pharmacy. Once the evolocumab 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 ornage 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 tready 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.

4.1 Evolocumab storage
The evolocumab prefilled SureClick autoinjectors are stored in the original carton and are kept in refrigerated storage. Allowed temperature excursions while in storage is 36°F to46°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.

5 DATA MANAGEMENT

5.1 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.

5.2 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.

6 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. The study comprises of 2 periods: (1) high-dose (HD) statin therapy and (2) HD statin + evolocumab therapy. Subjects will be on a HD statin therapy for 8 weeks followed by the initiation of evolocumab 420mg Q 4 weeks x 2 doses, in addition to HD statin therapy for another 8 weeks. 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).

The study requires 5 clinic visits. Each clinic visit (except visit 4) includes laboratory measurements, safety assessment, and health and concomitant medication review. A complete physical exam and safety laboratory assessment are required for visits 1 and 2.

6.1 Laboratory measurements

Laboratory measurements (see section 2.2) will be obtained at baseline (prior to study-recorded/initiated 8 week HD statin therapy), end of 8 weeks HD statin therapy, 24 hours after first evolocumab (420mg Q 4 weeks) dose, and at end of study (after 8 weeks of HD statin + evolocumab therapy). The study will require clinic visits during the required laboratory measurements.

6.2 Health and concomitant medication review

During the follow-up clinic visits, changes in participant's medical condition (any new diagnosis, scheduled surgical procedures, etc) and concomitant medications should be updated. Eligibility will be re-confirmed, adverse events reported, side effects and symptoms assessed.

6.3 Physical Exams

A physical exam will be conducted by the investigator or research nurse during the screening period after obtaining consent to evaluate the general status of the subject and to further elucidate patient symptoms, risk factors, or concerns that may increase the subject's risk for adverse reactions to the study treatment. A physical evaluation may be conducted as a nursing assessment if a physical exam was performed as standard of care ≤1 month prior to screening or at visit 2 if no AE/SAE's have occured.

6.4 Safety assessments

Safety assessments will consist of monitoring and recording of adverse events and serious adverse events (see section 8). 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 after screening and initation of the HD statin.

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

6.5 Treatment compliance

Compliance will be assessed by the investigator and/or study personnel at visit 2 and end of study visit. Pill counts will be used to assess compliance with HD statin therapy. This information should be captured in the source document at each visit.Non-compliance is defined as <80%.

6.6 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 and administration, food-drug/drug-drug interactions, pregnancy/lactation warning, and when to call investigator/physician. Subjects will be instructed to inform physicians and dentists of study treatment intake and to list all prescription medications, over-the-counter medications, or dietary supplements they are taking or plan to take so study personnel knows about other treatments that may affect adverse event risk.

Table 1

Schedule of Events

	V1	V2	V3	V4	V5/EOS
	Screening/ Initiate HD statin therapy	**1st dose of evolocumab 8 weeks post HD statin therapy ± 5 days**	**24±4 hours post 1st dose of evolocumab**	**2st dose of evolocumab 4 weeks (±3days) post 1st dose**	**8 weeks (±5days) post 1st dose of evolocumab**
Informed Consent	X				
Inclusion/Exclusion Criteria	X				
Height and Weight	X				
Vital Signs (HR, BP)	X	X	X	X	X
Medical History	X	X	X	X	X
Review Prior/Concomitant Medications	X	X	X	X	X
Safety Labs (CMP and CK)	X[1]	X			**X**
Urine Or Serum Pregnancy Test[2]	X				X
Ankle/Brachial Index	X	X			X
Physical Exam	X[3]	X[3]			
Laboratory Measurements – Blood Sampling	X	X	X		X
Laboratory Measurements- Urine Sampling	X	X			X
Dispense Study Drug(s)	X	X		X	
Assign/Continue HD Statin Therapy	X	X	X	X	
Administer Evolocumab 420mg/Q4 wk x 2 doses		X		X	
Drug Accountability		X		**X**	X
Adverse Events	X	X	X	X	X

[1] CMP laboratory results within 3 months of Visit 1 are acceptable. If not available, a blood sample for CMP will be obtained and results reviewed prior to HD statin therapy initiation.

[2] Urine or blood specimen for pregnancy test will be collected on women of childbearing potential at screening visit and end of study.

[3] May be conducted as a nursing assessment if a physical was performed as standard of care ≤1 month prior to screening or at visit 2 if no AE/SAE's have occurred.

7 TIMING OF ASSESSMENTS

7.1 Visit 1: HD statin therapy

7.1.1.1 Subject demographic and baseline characteristics

Demographic data to be collected on all subjects include: date of birth, age, sex, race, ethnicity. Height, weight, blood pressure, heart rate, relevant medical history/current medical condition present before signing informed consent, diagnoses and not symptoms will be recorded. Lipid and other CV medications will be recorded in eCRFs.

- Obtain safety laboratory test results (comprehensive metabolic panel and CK) to check for renal and hepatic function prior to starting/continuing HD statin therapy. Urine or serum pregnancy test will be performed for women of childbearing potential only.
- Laboratory measurements (see 2.2, blood and urine sampling) will be obtained prior to initiation of study-HD statin therapy
- Perform complete physical examination or nursing assessment if applicable
- Measure ankle/brachial index
- HD statin therapy

  Eligible subjects may start HD statin therapy once it is confirmed that they meet all inclusion criteria and none of the exclusion criteria. Enrolled subjects for this study can include subjects who are statin-naïve or already on statin. Statin-naïve subjects will be started on HD statin therapy. HD statin therapy allowed for the duration of the study includes (a) atorvastatin 40mg and 80mg and (b) rosuvastatin 20 to 40mg. Please see below for HD statin therapy guidelines:
  - If not on statin→start HD statin therapy (atorvastatin 40mg or rosuvastatin 20mg as according to investigator discretion)
  - If on atorvastatin 40 mg once a day→continue
  - If on lovastatin, pitavastatin, simvastatin, or pravastatin→change to atorvastatin 40 mg once a day
  - If on atorvastatin 80mg once a day→continue
  - If on rosuvastatin 20 to 40mg once a day→continue
  - If on rosuvastatin 10mg once a day→increase to 20mg once a day
- Provide patient education and medication instructions form for HD statin use
- Drug Accounatbility of statin therapy

7.2 Visit 2: HD statin + evolocumab 420mg Q 4 weeks therapy

7.2.1.1 Subjects will return to clinic for lab measurements (see section 2.2, blood and urine sampling) after 8 weeks (± 5 days) of HD statin therapy. Lab measurements will be obtained prior to first dose administration of evolocumab therapy.

7.2.1.2 Assessment of treatment compliance (i.e. HD statin therapy)

7.2.1.3 Perform complete physical examination

7.2.1.4 First dose administration of evolocumab 420mg

7.2.1.5 Provide patient information hand-out for study drug. Second dose of study drug-evolocumab will be 4 weeks after first dose. Subject will continue assigned HD statin therapy.

7.2.1.6 Safety laboratory test

7.2.1.7 Health and medication review

7.2.1.8 Adverse events assessment

7.2.1.9 Measure ankle/brachial index
7.2.1.10 Collect vital signs (HR, BP)

7.3 Visit 3: 24 hours post first dose of evolocumab

7.3.1.1 Subjects will return to clinic 24 ±4 hours post first dose of evolocumab for lab measurements (see section 2.2.1)
7.3.1.2 Adverse events assessment
7.3.1.3 Health and medication review
7.3.1.4 Collect vital signs (HR, BP)

7.4 Visit 4: HD statin + evolocumab 420mg continue Q 4 weeks therapy

7.4.1.1 Subjects will return to clinic after 12 weeks (± 3 days) of HD statin therapy.
7.4.1.2 Assessment of treatment compliance (i.e. HD statin therapy)
7.4.1.3 Second dose administration of evolocumab 420mg
7.4.1.4 Adverse events assessment
7.4.1.5 Health and medication review
7.4.1.6 Collect vital signs (HR, BP)

7.5 End of study visit

7.5.1.1 Subjects will return to clinic 8 weeks (±5 days) post first dose of evolocumab for lab measurements (see section 2.2.1, blood and urine sampling).
7.5.1.2 Adverse events assessment
7.5.1.3 Safety laboratory tests
7.5.1.4 Health and medication review
7.5.1.5 Assessment of treatment compliance (HD statin therapy)
7.5.1.6 Urine or serum pregnancy test for women of childbearing potential
7.5.1.7 Subjects to continue original statin therapy
7.5.1.8 Measure ankle/brachial index
7.5.1.9 Collect vital signs (HR, BP)

8 ADVERSE EVENTS

8.1 Adverse Event (AE) Definitions

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

A suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. A suspected adverse reaction implies a

lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

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

8.2 Serious Adverse Event (SAE) Definition

An SAE is defined as an event that:

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

8.3 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.

8.4 AE 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.

8.5 Procedures for Recording and Reporting of Adverse Events

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

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

8.6 Reporting of Pregnancies

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.

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 or serum pregnancy test prior to initiation of HD statin therapy (screening visit) and will undergo urine or serum 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. No further doses of IP would be administered in these cases.

8.7 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.

9 SUBJECT COST AND FUNDING

This is an investigator initiated study funded by Amgen Inc. Amgen will provide evolocumab.

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. Subjects will be compensated $40 per visit ($200 for entire study).

10 CONFLICTS OF INTEREST

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

11 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 necessary for subject assessment.

12 OUTSIDE CONSULTANTS/COLLABORATORS

There are no outside consultants/collaborators participating.

10 CONTRACTURAL AGREEMENTS

There are no outside consultants/collaborators participating.

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