Plaque Regression and Progenitor Cell Mobilization with Intensive Lipid Elimination Regimen (PREMIER)

Version 2.4 (Pivotal Study)

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1. Rationale

1(a) Background

The most intensive pharmacologic lipid lowering therapy with statins, though proven superior to standard dose regimens, is still associated with an unacceptably high rate of recurrent cardiovascular (CV) events early after an acute coronary syndrome (ACS). Progression or rupture of lipid rich necrotic core (NC) elements of atherosclerotic vulnerable plaque (VP) leads to a majority of recurrent CV events. Vascular healing by endothelial progenitor cells (EPC) plays a crucial role in repair following ischemic injury primarily by endothelialization of VP and neovascularization of ischemic myocardium. In fact, EPC mobilization while on statin therapy has been shown to enhance coronary blood flow in patients with stable coronary artery disease (CAD), and reduce myocardial ischemia and CV events in patients with ACS within a few weeks of treatment. This has prompted a continuous drive towards lowering of total cholesterol and specifically low-density lipoprotein (LDL). However, what still remains uncertain is whether the most intensive LDL-lowering therapy (ILLT) with LDL-apheresis could lead to a rapid and detectable reduction in VP atheroma volume, along with a more robust EPC mobilization compared to standard statin therapy in ACS patients.

1(b) Hypothesis

We hypothesize that in ACS patients undergoing percutaneous coronary intervention (PCI), ILLT with LDL-apheresis plus statin therapy will significantly reduce the total atheroma volume of VP and augment mobilization of peripherally circulating endothelial progenitor cell colony forming units (EPC-CFU/ml), compared to guideline based standard statin monotherapy alone (SMT). We also hypothesize that the total number of and the percentage of patients with major peri-PCI procedure adverse events will not be a safety concern for the study.

1(c) Specific Objectives

This is the second phase of a multi-center trial of ACS patients. The first phase was a safety study involving 31 patients and was completed in December 2012. US Food and Drug Administration (FDA) reviewed the safety and efficacy data from the first phase and approved proceeding to this phase. In this trial, 128 participants will be randomized in a 1:1 ratio to either initial LDL-apheresis and an oral daily dose of 40-80mg of Atorvastatin or equivalent (ILLT group) vs. a daily dose of 40-80mg of Atorvastatin or equivalent without LDL-apheresis (SMT group) following an uncomplicated PCI. Intravascular ultrasound with virtual histology (IVUS-VH) derived coronary atheroma volume and composition will be obtained at baseline and 90 days after enrollment, while peripheral blood sampling will be performed at enrollment, and at 24 hours, 30 days, and 90 days post-PCI, along with a four-month and a six-month clinical follow-up visits to determine whether the ILLT group will affect:

1. The total number of and percentage of patients with major peri-PCI procedure adverse events *(primary safety endpoint)*

2. The total number of and percentage of patients with statin-related abnormal liver function test events and statin-related muscle injury events *(secondary safety endpoint)*
3. The total atheroma volume within a ≥ 20 mm long segment of the target coronary artery at 90 days IVUS-VH follow-up *(primary effectiveness endpoint)*
4. The %NC component within a ≥ 20 mm long segment of the target coronary artery at 90 days IVUS-VH follow-up *(secondary effectiveness endpoint)*
5. The EPC-CFU/ml of peripheral blood, compared to SMT group from baseline to 30 days and 90 days post-PCI *(secondary effectiveness endpoint)*
6. The major adverse CV events (MACE) at 90 days and at six months follow-up *(secondary effectiveness endpoint)*

2. Background and Significance

2(a) Background

2(a)(i). Lowering blood cholesterol with statins is well established as a long-term strategy to reduce death and ischemic CV events in patients with stable CAD[1-3]. Major mechanisms by which lipid lowering is thought to improve outcome include preventing the development of new atherosclerotic lesions and stabilizing existing atherosclerotic plaques[4]. In addition, statins can reduce vascular inflammation[5], decrease platelet aggregability and thrombus deposition[6], and increase endothelium-derived nitric oxide production[7]. Most recently, statins have been reported to promote the neovascularization of ischemic tissue in normocholesterolemic animals by up regulating EPC mobilization and differentiation[8].

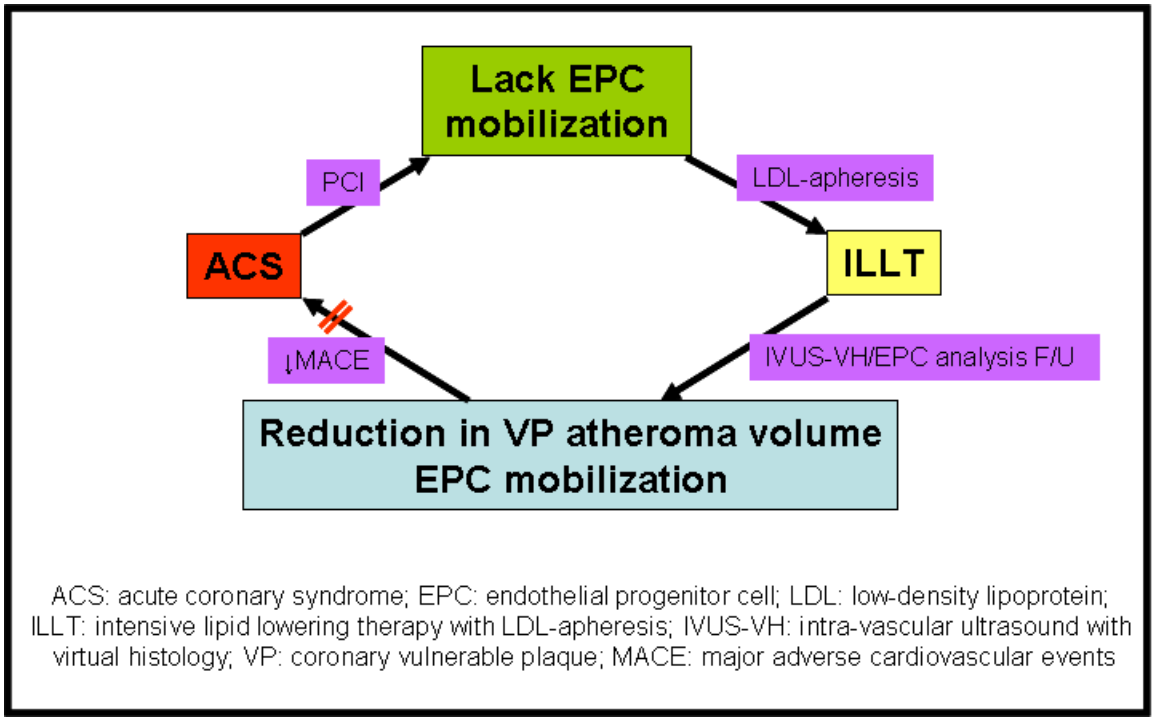
2(a)(ii). Aggressive lipid lowering pharmacotherapy with statins, though proven superior to standard dose regimens, still is associated with an unacceptably high rate of recurrent CV events (28.8%)[1,9]. Moreover, intensive statin regimens are significantly underutilized (5%) in ACS patients undergoing PCI, who are at highest risk for recurrent ischemic events[10]. ACS patients, who survive through the presentation, have a high rate of recurrent ischemic events, early after an index event[3]. This has prompted a continuous drive towards lowering of LDL goals.

2(a)(iii). Current analysis of statin studies also cannot definitively determine whether the benefit seen with high-dose statins is because of the high statin dose used or because low LDL levels are achieved. Our study will provide crucial information in this area by comparing ILLT which achieves a rapid drop in LDL levels using initial LDL-apheresis, followed with 40-80mg daily Atorvastatin or equivalent to SMT with 40-80mg daily Atorvastatin or equivalent. This has particular relevance in the context of our recent publication that showed ACS patients lack the ability to mobilize EPC in response to an endovascular injury[11]. Thus, up-regulation of EPC mobilization with ILLT in ACS patients may define a novel mechanism **(Figure 1)** and therapeutic strategy to improve neovascularization of ischemic myocardium, and endothelialization of VP and endovascular stent prosthesis, especially drug-eluting stents. Circulating EPC levels also correlate to CV risks and predict CV events[12].

2(a)(iv). Clinical studies like the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) trial have demonstrated that intensive cholesterol lowering with Atorvastatin administered immediately after hospitalization for ACS rapidly reduced the incidence of recurrent ischemic events over the first 16 weeks[13]. The Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis in Myocardial Infarction 22 (PROVE IT–TIMI 22) trial demonstrated that among patients who have recently had an ACS, an intensive lipid-lowering statin regimen provides greater protection against death or major CV events than does a standard regimen[9]. It showed a 16% reduction in the hazard ratio favoring intensive therapy. These early clinical benefits have been accompanied by only modest changes in intra-coronary plaque burden and are thought to be due to the atheroprotective property of mobilized EPC[14,15]. This early effect of statin treatment on clinical endpoints can be partly

explained by a 1.5-fold increase in the number of circulating EPC within one week after initiation of statin treatment, followed by sustained increased levels to three-fold throughout the four-week study period in patients with stable CAD[16]. There are currently no studies of EPC mobilization in ACS patients with intensive lipid lowering therapy.

Figure 1: Proposed Study Mechanism



ACS: acute coronary syndrome; EPC: endothelial progenitor cell; LDL: low-density lipoprotein; ILLT: intensive lipid lowering therapy with LDL-apheresis; IVUS-VH: intra-vascular ultrasound with virtual histology; VP: coronary vulnerable plaque; MACE: major adverse cardiovascular events

2(a)(v). Coronary atherosclerosis imaging: Although trials using clinical endpoints are the gold standard for evaluating novel atherosclerosis treatments, they are lengthy and resource-intensive. Surrogate endpoints, such as coronary angiography and IVUS-VH, allow a more cost-effective evaluation of novel treatments. IVUS-VH is a novel technology which allows for the identification of discrete plaque components using radiofrequency backscatter data[17]. IVUS can visualize the coronary artery wall and measure atherosclerosis volume. The reliability and reproducibility of total atheroma volume by traditional grey scale IVUS have been well validated, and we have published the first report on reproducibility of repeated IVUS-VH measurements in a clinical practice setting[32]. We reported excellent agreement between the two pullback measurements for lumen area vessel area and plaque burden. The Spearman rank-order correlation coefficients were (0.96, 0.96, and 0.95) for lumen area, vessel area, and plaque burden, respectively. (The data are presented in section 3(d) under "Work Accomplished").

IVUS has already been used as an endpoint in long-term trials (REVERSAL, or Reversal of Atherosclerosis with Aggressive Lipid Lowering[18] and ASTEROID, or Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound-Derived Coronary

Atheroma Burden[19]) and in short-term IVUS follow-up trials with Apo-A1 Milano[20] and delipidated high density lipoprotein (HDL) treatments[21]. Currently there are two clinical studies that have evaluated early (≤12 week) IVUS derived coronary atheroma regression and have demonstrated significant regression from baseline with pre-beta HDL[21] and ApoA-1 Milano[20] infusions. The change in atheroma volume in the control arm was 2.8mm^3 (increase) and that in the treatment arms were -12.18mm^3 and -14.1mm^3 with pre-beta HDL and ApoA-1 Milano therapies respectively. We also expect a robust response to ILLT with LDL-apheresis at 90 days. Our estimates of effect have been described in detail in Section 4(g) under "Biostatistical Considerations."

2(a)(vi). Endothelial progenitor cells play a crucial role in repair following ischemic injury[22]. This is achieved primarily by neovascularization and endothelialization of vulnerable atherosclerotic plaque. Thus, augmentation of circulating EPC in the setting of an ACS may significantly contribute to endothelialization of coronary VP, and limit myocardial ischemic injury[22]. Increasing the number of circulating EPC by transplantation of hematopoietic stem cells or by injection of in vitro differentiated EPC has been shown to improve neovascularization of ischemic hind limbs[23], accelerate blood flow in diabetic mice[24], and improve cardiac function[25]. Therefore, augmentation of circulating EPC in the setting of an ACS may significantly contribute to the stimulation of neovascularization after tissue ischemia. Recently, EPC mobilization with statin therapy has not only been shown to rapidly enhance coronary blood flow in patients with stable coronary artery disease[26], but also to reduce myocardial ischemia in patients with ACS within a few weeks of treatment[27], along with a significant increase in circulating EPC. The current studies however, cannot definitively determine whether the EPC mobilization seen with statins is because of the statin dose used or the degree of LDL reduction achieved.

2(a)(vii). LDL-apheresis is an extracorporeal blood processing system to acutely remove LDL-cholesterol from the plasma[28]. The approved LIPOSORBER® system will be used for the study. It contains dextran sulfate cellulose beads that selectively bind Apo-B containing lipoproteins (Lp(a), LDL and VLDL). During this procedure, plasma is separated from whole blood, LDL cholesterol removed from the plasma, and plasma and blood cells are recombined and returned to the patient[28]. LDL-apheresis treatment can lower LDL cholesterol levels by 85% after a single treatment. In familial hyperlipidemia (FH) patients, this significant lowering of the LDL cholesterol level is not maintained due to a metabolic defect that causes the overproduction of LDL cholesterol. The LDL cholesterol level begins to increase (or rebound) after treatment, eventually returning to baseline in about one to three weeks, requiring recurrent treatments[28]. We do not expect non-FH ACS patients to rebound rapidly, especially on continued statin therapy. We therefore propose using only one initial treatment with LDL-apheresis to achieve a rapid reduction in LDL-cholesterol, with follow-up guideline based lipid lowering pharmacotherapy with statins.

LIPOSORBER® treatment is very selective in removing LDL cholesterol, and does not affect HDL and triglyceride (TG) levels. A mean reduction in plasma proteins (15%), fibrinogen (29%), and platelets (5%) may be observed and rarely pose a risk to the patient[28,29]. Approximately 300,000 treatments with the LIPOSORBER® system

have been performed worldwide on over 2,500 patients. Possible adverse reactions include hypotension, nausea/vomiting, flushing, chest pain, fainting, lightheadedness, anemia, abdominal discomfort, numbness/tingling, tachycardia, headache, shortness of breath, hemolysis, bradycardia, itching/hives, bleeding (due to the use of unfractionated heparin) and chills[28]. Hypotension is the most common, and in US trials, occurred in <1% of all treatments and was treated effectively with intravenous fluids. If an ACE (angiotensin converting enzyme)-inhibitor is used to manage the patient's blood pressure; it should be held 36 hours prior to LDL-apheresis.

A single treatment with LDL-apheresis has been demonstrated to have significant effect on arterial blood flow. Tamai et al. demonstrated that a single LDL-apheresis treatment increased nitrous oxide production and forearm blood flow[29]. A single treatment with LDL-apheresis has also been shown to significantly reduce modified LDL particle, remnant-like particle-cholesterol, and C-reactive protein[30]. In the LACMART study, one LDL-apheresis treatment over one year lowered the LDL of FH patients aggressively and demonstrated a significant reduction in the plaque area and increase in the minimum luminal diameter (MLD) with an annual angiographic and IVUS follow-up[31]. It also demonstrated that the benefits of LDL-apheresis are substantial when used in the setting of a statin pharmacotherapy. It is important to point out that in the FH population, the LDL cholesterol level in the medication-only arm did not change significantly from baseline (174±39mg/dl to 181±53mg/dl), and with LDL-apheresis it decreased from 213±25mg/dl to 140±27mg/dl, which is much higher than the expected post-LDL-apheresis LDL levels expected in non-FH ACS patients. The details of atheroma regression are described in section 4(g), “Biostatistical Considerations”.

Thus, we believe that the proposed ILLT strategy to jumpstart aggressive LDL reduction post-ACS is expected to have significant pathophysiologic and biochemical effects, in addition to significant atheroma regression expected with nearly 85% reduction in serum LDL levels. Though the vasoactive effects of LDL-apheresis are well known, its effect on EPC mobilization has never been studied. Our proposal will for the first time test this hypothesis and lay the foundation for a dedicated mechanistic study in the near future.

2(b) Relevance to Veterans Health

We believe that our proposed strategy would provide the basis for a large-scale study to evaluate clinical endpoints as a primary objective and ultimately lead to the development of a new strategy to attain more aggressive therapeutic lipid goals, and reduce CV events post-ACS, reduce the socioeconomic burden of ischemic heart disease, and improve the quality of life of our veterans.

3. Work Accomplished

This proposal is a logical extension of our ongoing work. We have:

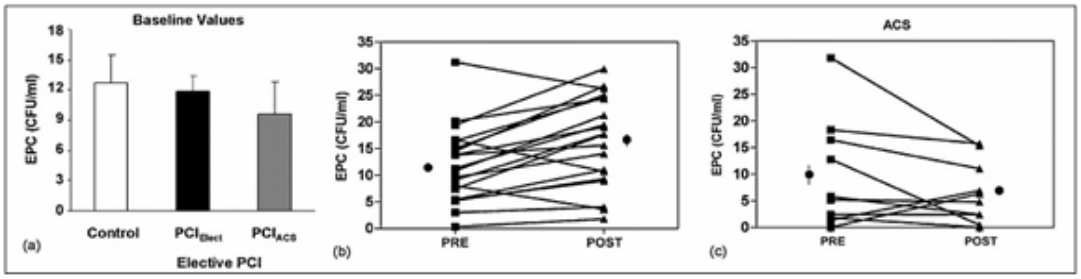
i. Reported for the first time, failure of ACS patients to mobilize EPC in response to PCI in contrast to those with stable coronary lesions who demonstrate a robust EPC response to endovascular injury
ii. Successfully isolated and identified EPC-CFU from peripheral blood samples of patients undergoing PCI
iii. Performed LDL-apheresis in patients with CAD
iv. Performed and reported IVUS-VH validation study to detect coronary atheroma volume and its components
v. Demonstrated clinical trial experience by completing and publishing the SOS (Stenting of Saphenous vein graft) multi-center randomized trial with 12-month angiographic and IVUS follow-up

3(a) EPC Study in ACS

We have reported that EPC levels rise in response to PCI in patients with stable coronary lesions. In contrast, patients with unstable coronary lesions or ACS failed to mobilize EPC in response to PCI **(Figure 2)**.

These data, the first in humans undergoing PCI, are consistent with a protective role for EPC mobilization. In this study, we investigated the effect of a discrete endovascular insult on human EPC recruitment into the peripheral circulation[11]. By focusing on a defined clinical procedure, we were able to compare EPC levels before, and early after the manipulation at reproducible time points, avoiding possible nonspecific perturbations.

Figure 2: Baseline EPC levels and mobilization after PCI



(a) Baseline (pre-catheterization) EPC levels from patients with acute coronary syndrome (PCIACS-group), elective percutaneous coronary intervention (PCIElectgroup), and normal epicardial coronary artery (control) patient groups. Baseline EPC-CFU levels were not different between groups (P > 0.05). EPC-CFU in PCIElect group (b) and PCIACS group (c) are shown before (PRE, squares) and after (POST, triangles) PCI. EPC-CFU levels increased in the PCIElect (P = 0.0009) but not in the PCIACS (P = 0.20) group. Circles are group means with S.E.M.

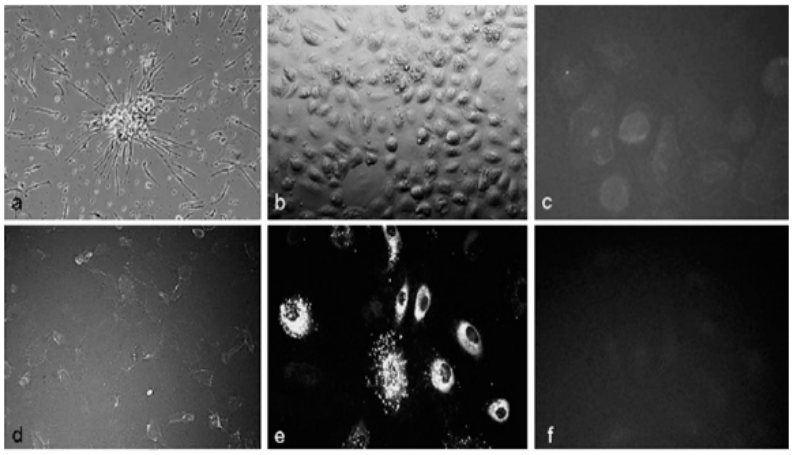
3(b) EPC Isolation

We have successfully isolated and identified EPC from peripheral blood samples of patients undergoing PCI using:

i. Cell culture technique **(Figure 3)**
ii. Flow-cytometry **(Figure 4)**

3(b)(i). EPC assay, using the cell culture method: EPC form typical colony forming units morphologically, and transform into cobblestone monolayer after 2-3 weeks **(Figure 3a-b).** Surface markers characteristic for EPC are von Willebrand Factor (vWF+), CD31+, CD34+, CD45- **(Figure 3c-f)**[11]**.**

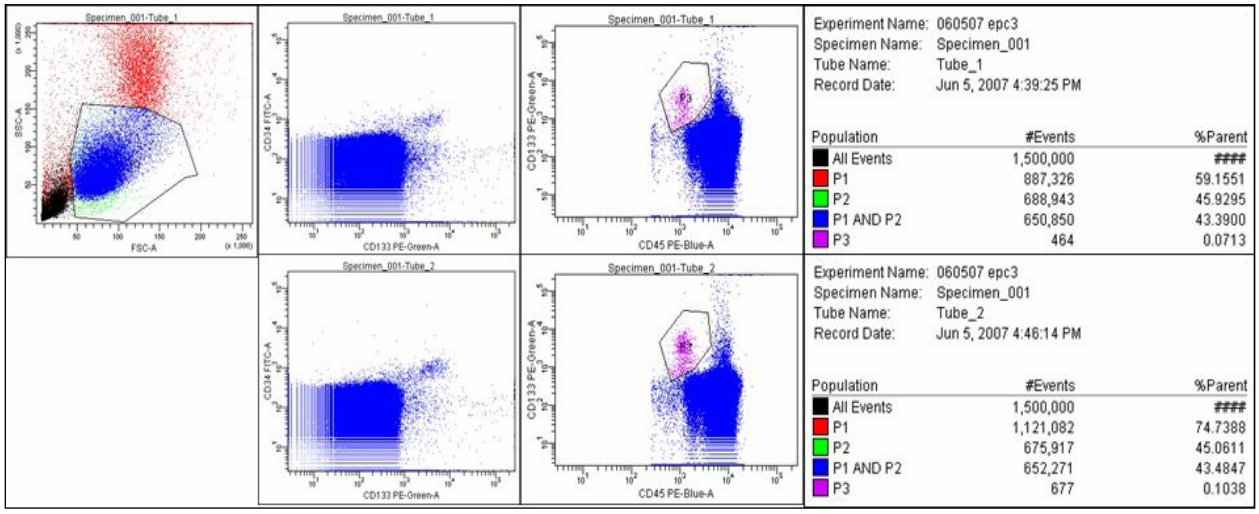
Figure 3 (a-f): Confirmation of EPC-CFU phenotype by cell culture



(a) Peripheral mononuclear cells cultivated for 7 days develop EPC-CFU demonstrating a typical central core of small round cells with elongated spindle cells at the periphery, (b) at 3 weeks, monolayers show cobblestone appearance. Photomicrograph obtained with Nomarski DIC. Indirect immunofluorescence endothelial lineage of these cells was confirmed by immunocytochemical staining for CD34+ (c), CD31+ (d), von Willebrand Factor + (e), and CD45 weekly+ (f).

3(b)(ii). Using flow cytometry, we have also measured and confirmed isolated EPC in peripheral blood samples. The results of such an analysis in two patients recently performed in our laboratory are shown below **(Figure 4),** and demonstrate CD34+, CD133+, and CD45 weakly+ circulating EPC, similar to those identified by cell culture technique **(**purple) (unpublished data).

Figure 4: Circulating EPC Levels by Flow Cytometry



3(c) LDL-apheresis in Patients with CAD

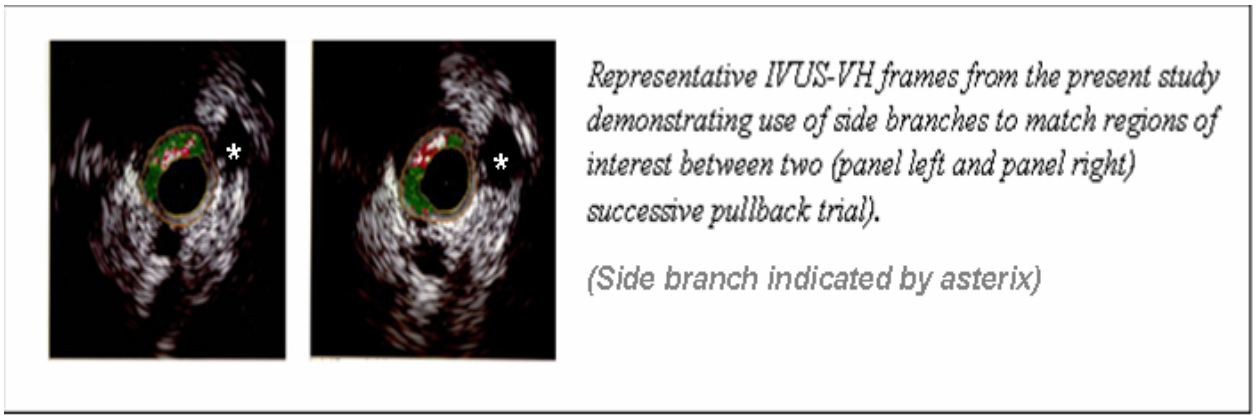
Our group, under the directorship of Dr. Ravindra Sarode (Director LDL-apheresis center, University of Texas Southwestern Medical Center, Dallas, TX), has performed 12 uneventful LDL-apheresis sessions in two patients with CAD. One patient underwent LDL-apheresis every week, and the second every two weeks. Average reduction of LDL was approximately 84% after each session. Both patients have also been maintained on low dose statin therapy.

From our experience in the first phase of the PREMIER trial, there was one transient hypotension episode recorded in 20 patients randomized to LDL-apheresis after PCI for ACS. The event resolved spontaneously and was attributed to micturition.

3(d) Identification of Coronary Atheroma with IVUS-VH in ACS Patients undergoing PCI

The principal investigators (PI's) perform over 2,300 cardiac catheterizations and 500 coronary interventions annually, of which >80% are IVUS-VH guided. Coronary angiographic image acquisition, quantitative coronary angiographic analysis (QCA) using the Phillips proprietary software is performed by the PI's. The quantitative IVUS-VH image acquisition, live measurements of vessel diameter, cross-sectional area, plaque volume, and plaque composition assessments (QIVUS) are made by the PI's[32]. **Figure 5** demonstrates typical QIVUS assessments and measurements made during a typical study. These procedures can be performed reproducibly and safely by the PI's. The overall annual complication rate of our cardiac catheterization laboratory is 1.6%, of which 85% consist primarily of minor access site complications.

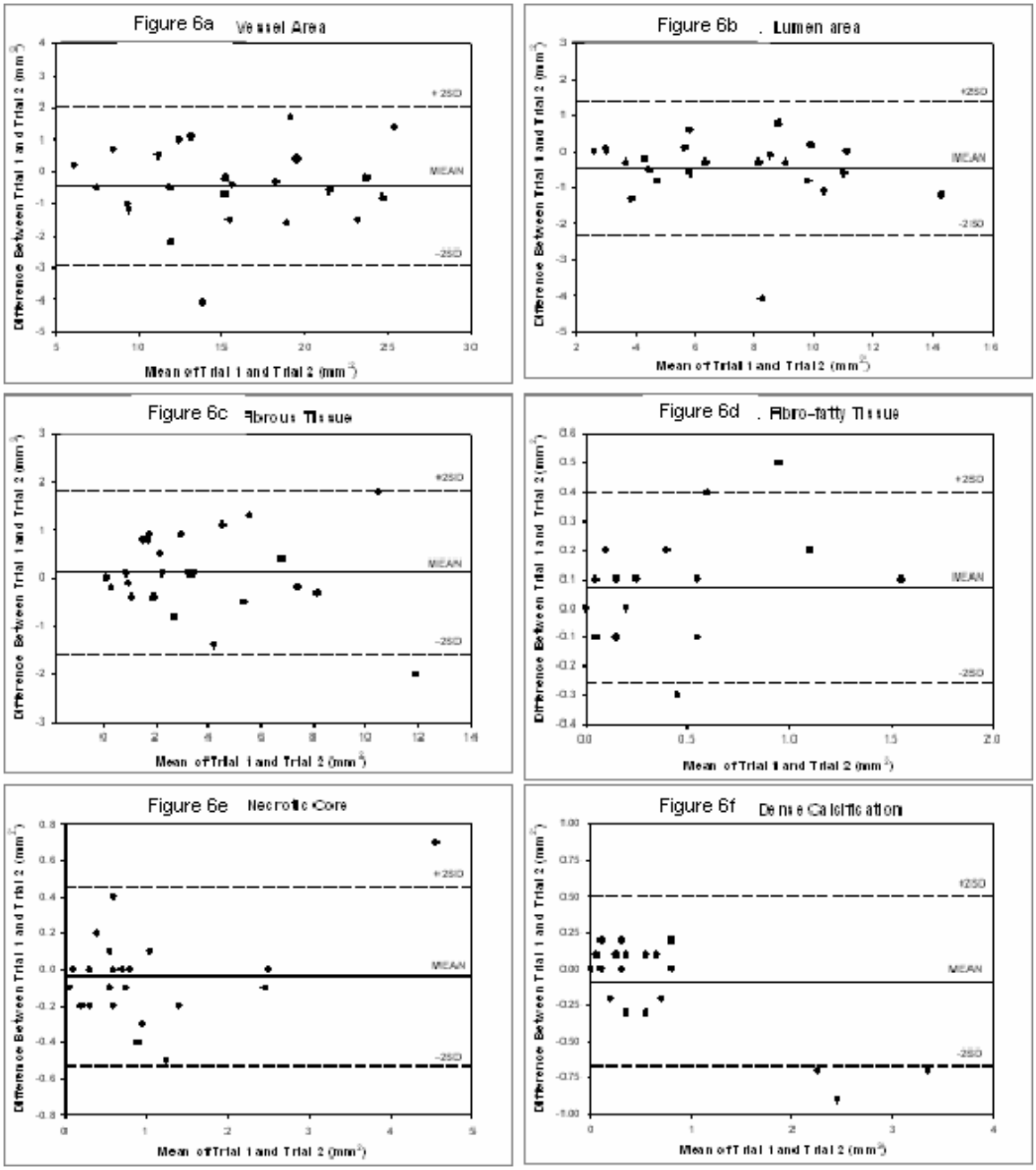
Figure 5: Representative IVUS-VH frames from the present study demonstrating use of side branches to match regions of interest between successive pullback trials



The reliability and reproducibility of total atheroma volume by traditional grey scale IVUS has been well validated, and the PI's have published the first report on reproducibility of repeated IVUS-VH measurements in a clinical practice setting[32]. We reported excellent *Bland-Altman plots demonstrating reproducibility data for mean vessel area (a), lumen area (b), fibrous tissue (c), fibrofatty tissue (d), necrotic core (e), and dense calcification (f), respectively,* agreement between the two pullback measurements for lumen area vessel area, and plaque burden (data not shown). The Spearman rank-order correlation coefficients were (0.96, 0.96, and 0.95) for lumen area, vessel area, and plaque burden respectively.

VH analysis Spearman rank-order correlation coefficients between pullback trials for the fibrous, fibrofatty, necrotic core, and dense calcium measurements were (0.97, 0.90, 0.90, 0.90) respectively and indicated a high level of reliability (data not shown). Coefficients ranged from 0.90 to 0.97 ($p<0.0001$). The Bland-Altman plots **(Figures 6a-f, above)** indicated proportional error for the differences of the four measurements between the pullback trials. Accordingly, the CR values were less than $z=1.96$, ranging from 0.33 to 1.66.

Figures 6(a-f): Bland-Altman plots indicated proportional error for the differences of the four measurements between the two pullback trials



4. Work Proposed

The study was proposed to be carried out in two phases. The first phase was a safety study, in which 31 ACS patients were randomized to either ILLT (device) group or SMT (control) group with a randomization scheme of 2:1 (20 ILLT patients and 10 SMT patients). After all 30 out of 31 randomized ACS patients in the first stage completed the 6-month follow-up visit, the study data (for both safety and efficacy) was submitted to FDA for review. FDA approved continuation to the second phase for the pivotal study, which will include 128 ACS patients randomized to either ILLT (device) group or SMT (control) group with a randomization scheme of 1:1 (64 in ILLT device group and 64 in SMT control group).

4(a) Study Objectives

The safety objective of this study is to collect additional safety data for ACS patients who undergo the LDL-apheresis post-PCI procedure and the use of maximum dose of statin drugs.

The primary effectiveness objective of this study is to compare the total atheroma volume within a ≥ 20 mm long segment of the target coronary artery in ACS patients with an uncomplicated PCI randomly assigned to LDL-apheresis with 40-80mg daily of Atorvastatin or equivalent (ILLT group) or 40-80mg daily of Atorvastatin or equivalent only (SMT group). The specific hypothesis being tested is: ILLT is superior to SMT in reducing the total atheroma volume at 90 days IVUS-VH follow up.

The secondary effectiveness objectives of this study will be: (1) to compare %NC component of atheroma within a ≥ 20 mm long segment of the target coronary artery; (2) to compare the EPC-CFU/ml of peripheral blood in the two treatment groups; and (3) to compare MACE, associated with the two treatment groups at 90 days and at 6 month follow-up.

Rationale for study effectiveness objectives: The rationale for the study effectiveness objectives includes the observation of increased recurrent CV events in ACS patients early (three months) post-index event, despite intensive statin pharmacotherapy, due to progression and rupture of non-critical coronary lesions. Aggressive LDL lowering using LDL-apheresis (ILLT) as proposed in this study will test a novel strategy of non-critical coronary atheroma regression at 90 days, and hence reduce early (12-week) MACE events in ACS patients, sustained at end of study (at least six months follow-up). We also propose to test whether EPC mobilization with aggressive LDL lowering correlates with coronary atheroma regression, change in atheroma composition (%NC), and MACE events. We acknowledge that the scope of this study does not test the mechanism of EPC targeting to site of coronary VP and its endothelialization, however, it would provide for the first time important initial evidence which could be tested in future dedicated studies.

We expect to demonstrate a significant reduction in atheroma volume at 90 days by IVUS-VH (primary endpoint) because this study will have the most aggressive LDL lowering strategy in ACS patients ever tested in non-FH ACS patients. The 12-week time period is also the time point when early post-ACS CV events begin to increase, as seen in the PROVE IT–TIMI 22 trial[9]. Currently there are two clinical studies that have evaluated early (≤12 week) IVUS derived coronary atheroma regression and have demonstrated significant regression from baseline with pre-beta HDL[21] and ApoA-1

Milano[20] infusions. The change in atheroma volume in the control arm was 2.8mm^3 (increase) and that in the treatment arms were -12.18mm^3 and -14.1mm^3 with pre-beta HDL and ApoA-1 Milano therapies respectively. We also expect a robust response to ILLT with LDL-apheresis at 90 days. Our estimates of effect are much more conservative and have been described in detail in Section 4(g) under "Biostatistical Considerations".

4(b) Study Outcome Measures

The primary safety outcome measures will be the total number of and percentage of patients with major peri-PCI procedure adverse events, such as hypotension, angina, myocardial ischemia, myocardial infarction (if the patient is determined to have had unstable angina rather than non-ST-elevation MI at admission), cerebrovascular event (CVA), vermicular tachycardia, bleeding (at the PCI access and apheresis cannulation sites in the apheresis patients and at the PCI access site in the non-apheresis control patients), and death. The peri-PCI procedure is defined as encompassing the time of the PCI procedure and the time of the subsequent LDL-apheresis procedure for ILLT group vs. the time of the PCI procedure for SMT group. All LDL-apheresis-related adverse events in the ILLT group, including any minor expected events, will be recorded.

The secondary safety outcome measures will be the total number of and percentage of patients with statin-related abnormal liver function test events and statin-related muscle injury events, which could occur due to the maximum dose of statin drugs being given to both the ILLT group and SMT group patients. The statin-related muscle injury is defined as a muscle injury which cannot be attributed to a non-statin cause and which is evidenced by symptoms (muscle soreness, pain, or tenderness) and/or lab tests such as serum total creatine phosphokinase (CPK), CPK-MM, or myoglobin.

The primary effectiveness outcome measure will be the change in the total atheroma volume within a ≥ 20 mm long segment of the target coronary artery from baseline to 90 days post-PCI. The measurement will be done via IVUS-VH at both centers of investigation.

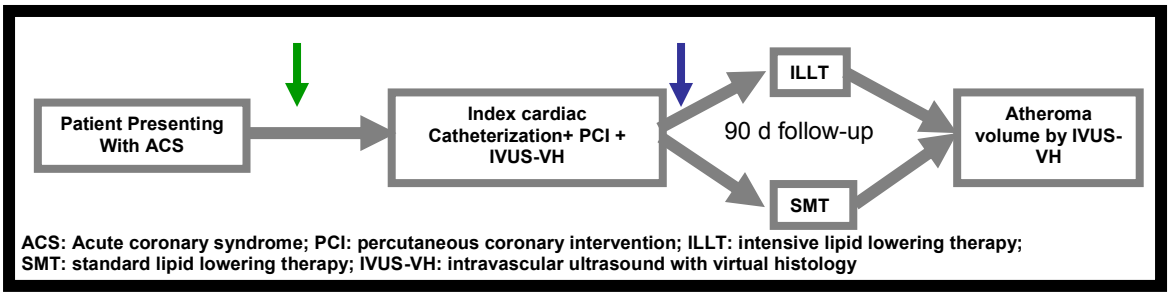
The secondary effectiveness outcome measures will include the %NC component of atheroma, EPC-CFU/ml of peripheral blood, and major adverse cardiovascular endpoints (MACE) including death, myocardial infarction, coronary revascularization, and stroke during the follow-up periods. The cell culture assay and quantification of circulating EPC-CFU will be done for patients recruited at the Dallas VA center. The major adverse CV events will be collected via both clinical visit and searches of the Austin database for VA hospital utilization by Hines staff. Approvals for using the patients' Social Security numbers for data searching will be obtained.

4(c) Study Design and Procedures

The proposed study is a multi-center, randomized controlled study comparing atheroma volume, %NC component, and peripherally circulating EPC-CFU/ml of blood in ILLT vs. SMT groups of ACS patients undergoing PCI **(Figure 7).** All patients will sign an informed consent during the index hospital admission for ACS but prior to any study-

related procedure. The protocol will be approved by each site's institutional review board.

Figure 7: Study design overview for primary endpoint:



4(c)(i). Subject Screening Criteria **(Figure 7, green arrow)**: Patients presenting with an ACS, LDL ≥70mg/dl on Atorvastatin ≤80mg or equivalent dose of another statin, normal liver function test (LFT) and referred for non-emergent cardiac catheterization (the procedure is not required to be performed within 3 hours after patient presentation) will be screened and consented during the index hospital admission for ACS. Patients on ACE inhibitors will either have it discontinued or switched to an angiotensin receptor blocking agent (ARB). All screen eligible patients will be subsequently asked to sign an IRB-approved informed consent form prior to clinically indicated PCI or any subsequent study related tests.

ACS is defined as chest discomfort lasting at least 10 min within the last 24h, new ≥lmm ST-wave or dynamic T-wave changes in at least two contiguous EKG leads but without significant ST elevation, and/or troponin I or T levels above the 99th percentile. Abnormal LFT is defined as any liver transaminases (ALT or AST) ≥ 3 times the upper limit of the normal laboratory reference. Atorvastatin 40-80mg will be considered equivalent to 20-40mg of Rosuvastatin. Patients on Simvastatin at the time of enrollment will be switched to Atorvastatin 40-80 mg or Rosuvastatin 20-40 mg.

After PCI of the target or culprit coronary artery (as determined by the operator) and IVUS-VH analysis of a ≥20mm segment of the target coronary artery are performed during the index hospitalization for the qualifying ACS event, the patients will be randomized to ILLT or SMT if they meet all inclusion and exclusion criteria **(Figure 7, blue arrow).** PCI will be performed using standard technique at the discretion of the operator. 40-80mg daily Atorvastatin or equivalent treatments (ILLT or SMT) will be initiated within 24 hours after randomization. Patients randomized to the ILLT arm will undergo LDL-apheresis within 36 hours after presentation unless the 36-hour window is mandatory for those who were on ACE inhibitors to allow for the complete washout of ACE inhibitors. Adjunctive anti-platelet and anti-thrombin therapies will be at the discretion of the primary operator and clinical team. Refer to **Figure 8** for all study timelines.

4(c)(ii). Inclusion Criteria:

(1). Willing and able to provide informed consent (including HIPAA)

(2). Age >30 years

(3). Presenting with acute coronary syndrome (ACS), manifested as unstable angina or non-ST-elevation myocardial infarction
(4). Referred for clinically indicated, non-emergent (the procedure is not required to be performed within 3 hours after patient presentation) coronary angiography and PCI with IVUS-VH of target coronary artery for ACS
(5). Successful placement of two large-bore IV cannulas in bilateral upper extremities
(6). Fasting (≥ 12 hrs) LDL ≥70mg/dl while on ≤80mg Atorvastatin or equivalent dose of other statin, performed at time of admission

4(c)(iii). Exclusion Criteria:
(1). Known allergy to aspirin, statins, or iodinated contrast
(2). Positive pregnancy test, planning to become pregnant, or breast-feeding
(3). Coexisting conditions that limit life expectancy to less than six months or affect patient compliance
(4). Uncontrolled fasting (≥ 12 hrs) triglyceride levels (≥500mg/dl)
(5). Already participating in an investigational device or drug study
(6). History of heparin induced thrombocytopenia (HIT)
(7). Persons with estimated GFR less than 45 ml/min
(8). ST-elevation myocardial infarction at admission
(9). Abnormal liver function test (LFT) at time of admission, with abnormal LFT defined as any liver transaminases (ALT or AST) ≥ 3 times the upper limit of the normal laboratory reference
(10). Pre-PCI or post-PCI left ventricular ejection fraction <25% by echo or cardiac catheterization done after admission
(11). Pre-PCI, intra-PCI, or post-PCI hemodynamic instability with hypotension
(12). Pre-PCI, intra-PCI, or post-PCI cardiac arrest
(13). Pre-PCI or post-PCI acute heart failure with or without pulmonary edema
(14). Intra-PCI or post-PCI sustained ventricular tachycardia
(15). Complicated PCI, defined as PCI with any of the vascular access complications (large hematoma with lump > 5 cm or requiring medical treatment; AV fistula; pseudo aneurysm requiring treatment; retroperitoneal bleeding), or PCI with any of the procedural complications (abrupt vessel closure; no-reflow phenomenon; new angiographic thrombus; new major dissection with reduced flow; catheter-related thrombus), or PCI requiring further medical treatments (urgent CABG; endotracheal intubation; unplanned in-aortic balloon pump; LVAD; covered stent; unplanned temporary pacemaker wire; administration of inotropes; CPR) , or PCI resulting in clinical events (death; stroke; myocardial infarction; stent thrombosis) during or within 24 hours after the index PCI
(16). Post-PCI ongoing chest pain
(17). Post-PCI severe groin pain and hematoma > 5cm in diameter

(18). Persons whose hemoglobin is less than 9 grams following the index PCI/IVUS procedure, or who experience a drop in hemoglobin of greater than or equal to 2 grams following the procedure
(19). Not able to comply with study protocol as determined by the investigators

4(c)(iv). Baseline Data Collection: A complete history and physical, 12-lead electrocardiogram, lipid panel, basic metabolic panel (BMP) with LFT, complete blood count, and complete cardiac catheterization, PCI, IVUS-VH and adjunctive pharmaco-therapy data will be collected on all eligible patients apart from demographic and contact information.

4(c)(v). Stratification and Randomization: Prior to randomizing a patient, all screening and baseline assessments must be completed and the patient determined to be eligible for inclusion in the study. Eligible patients will be randomized to one of two treatment groups (ILLT or SMT) following an uncomplicated index PCI**.** 40-80mg daily Atorvastatin or equivalent statin medication treatment (ILLT or SMT) will be initiated within 24h after randomization and LDL-apheresis for ILLT group will be initiated within 36 hours of hospital admission unless the 36-hour window is mandatory for those who were on ACE inhibitors to allow for the complete washout of ACE inhibitors. Refer to **Figure 8** for all study timelines. Screening and randomization will be performed by a study coordinator, and LDL-apheresis will be conducted by a contracted specialized apheresis nurse.

4(c)(v)(1). Randomization Methods: The randomization scheme and associated codes will be developed by the Cooperative Studies Program Coordinating Center (CSPCC). Permuted block randomization with random block sizes will be employed to assign patients to either ILLT or SMT in a 1:1 ratio. The randomization will be stratified by investigation sites only.

4(c)(v)(2). Randomization and Blinding Procedures: Following an uncomplicated PCI procedure, patients who are eligible and willing to participate in the study will be randomly assigned to either the ILLT or SMT arm using an automated telephone-based randomization system. A touch-tone phone is necessary to use this Voice Information System (VIS) to randomize eligible subjects. CSPCC will assign each site a unique 6-digit PIN that the unblinded staff can use to access the VIS system and randomize a subject (or add/update an SSN). The VIS will query the unblinded staff for subject information and confirm these data prior to determining the subject's treatment group assignment. A copy of the appropriately executed consent and HIPAA authorization documents must be on file at the Hines CSPCC within 24 hours of randomization. If the VIS is not working during normal business hours, the research coordinator can telephone the Hines CSPCC staff to randomize a patient. The subjects or Site Investigators will not be blinded to the study treatment assignment; however all follow-up visits and endpoint adjudications will be performed by a dedicated study nurse blinded to treatment allocation. Patients will be cautioned not to reveal his/her treatment allocation to the blinded study nurse. This blinding precaution will be emphasized by the

procedures written into the study Operations Manual and the communications to the Site Investigator, the Site Coordinator and other study personnel during the kick-off meeting and the teleconference calls throughout the study. Patients will be screened and consented before PCI, but will have the opportunity, after PCI, to decline participation in the study and will not be randomized if they choose not to participate.

4(d) Intervention and Treatment

The randomized treatment regimens of this study include:

(i) ILLT
(ii) SMT

4(d)(i): ILLT: Patients randomized to ILLT will undergo LDL-apheresis within 36h after presentation with an ACS unless the 36-hour window is mandatory for those who were on ACE inhibitors. All ILLT patients will also be started on Atorvastatin 40-80mg daily or equivalent within 24h after randomization. Patients will be continued on aspirin 81-325mg and clopidogrel 75mg daily. LDL-apheresis will be initiated in the intensive care unit or inpatient hemodialysis or plasmapheresis center, supervised by a dedicated contracted LDL-apheresis nurse and Dr. Banerjee and an apheresis expert. A hematologist or an apheresis specialist will supervise LDL-apheresis at the other study centers. Bilateral large bore brachial venous intravenous access will be obtained and the patient connected to the LIPOSORBER® machine. Pre-existing intravenous access sites can be used for this purpose to minimize patient discomfort associated with additional IV placements. Each machine has two apheresis columns. The system is primed with heparinized Lactated Ringer's injection, and 30-60 units of heparin per kilogram body weight is administered intravenously to the patient followed by an infusion throughout the treatment. When the first apheresis column has completed adsorbing LDL, the computer-regulated machine automatically switches the plasma flow to the second column to continue LDL adsorption. Simultaneously, the plasma remaining in the first column is returned to the patient. The first column is regenerated using a 5% Sodium Chloride Injection, eluting the LDL, VLDL and Lp(a). When elution is completed and flushed through the waste lines to a waste bag, the column is reprimed completely and ready for the next cycle of adsorption, allowing continuous LDL-apheresis. No additional fluids are given to the patient during column switchover, and only the treated plasma is returned. A typical procedure takes about three hours.

Typical LIPOSORBER® settings are:

Extracorporeal volume = approx. 400ml (170ml blood, 230ml plasma)
Plasma volume treated = approx. 0.7 x kg body weight x hematocrit
Treatment time = 2-4 hours
LDL-C acute reduction = 73-83%
Unfractionated heparin dose = 45 units/kg (of which 75% given as a single IV bolus dose at the initiation of LDL-apheresis, and the rest to be delivered via an IV infusion at the rate of 1 ml/hr [1000 units/ml formulation]). Heparin infusion to be stopped 30 minutes prior to the estimated end of treatment.

Machine settings ranges include:

Treatment volume = 0-9950ml plasma
Whole blood flow rate = 0-150ml/min
Plasma flow rate = 0-40% of whole blood flow rate
Heparin infusion rate = 0-5ml/hr
Blood warmer temp = 35-40°C

All patients randomized to ILLT will receive Atorvastatin 40-80mg daily or equivalent within 24 hours after randomization.

4(d)(ii). SMT: Standard medical therapy will include Atorvastatin 40-80mg daily or equivalent initiated within 24h after randomization. Patients will be initiated on Atorvastatin 40-80mg if their outpatient medication included Atorvastatin ≤80mg or equivalent dose of statin. Atorvastatin 40-80mg will be considered equivalent to Rosuvastatin 20-40mg. In the event of a restrictive formulary change of preferred statin drug within the VA system, the drug equivalency statement will serve as the reference for statin drug changes to meet the inclusion/exclusion and study follow-up LDL-goals as per the National Cholesterol Education Program (NCEP) and the latest ACC/AHA Lipid Lowering Therapy guidelines[33,37].

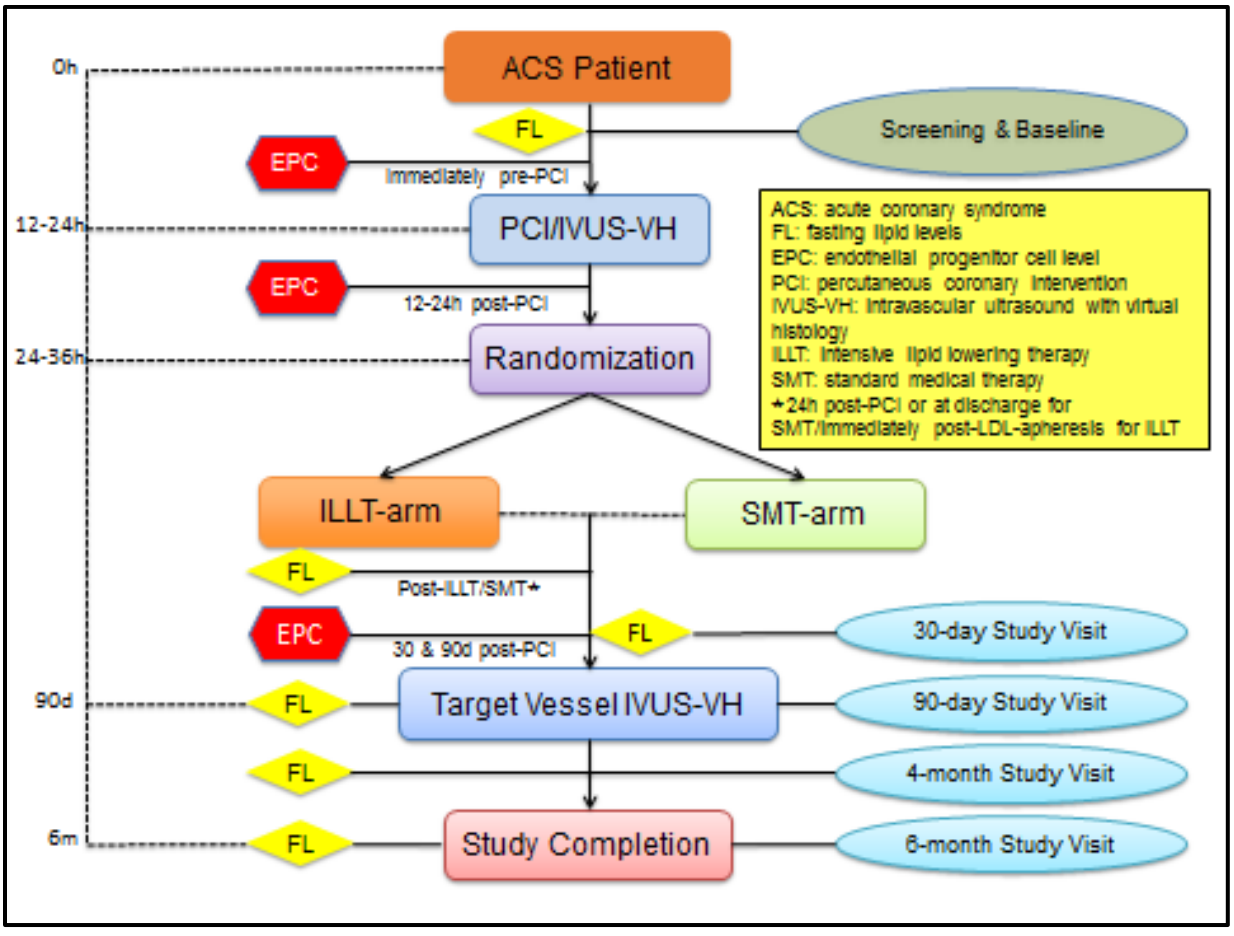
The study will not enroll patients with known history of allergy or contraindications to statin therapy. If a patient is shown to be intolerant to the treatment of Atorvastatin 40-80mg daily or equivalent after randomized into the study, the Site Investigator may use an alternative statin provided above (Simvastatin or Rosuvastatin). If the intolerance remains unresolved, the Site Investigator may adjust the doses or even discontinue the statin therapy per clinical needs. These patients will still be followed up and included in the final analysis based on the intent-to-treat principle.

4(d)(iii). Follow-up and study terminations: All participants will return for their scheduled study follow-up visits for six months. Participants will have the right to withdraw from the study at any time. However, Site Investigators may not withdraw a participant from the study without just cause and without the approval of the Study Chair.

4(e) Follow-up Assessments

All study patients will undergo IVUS-VH analysis at index PCI and at follow-up. A minimum distance of 10mm away from the PCI site is the preferred site to visualize the atheroma volume with IVUS-VH. Patients will return at 90 days from index PCI for repeat cardiac catheterization and ≥20mm matched target coronary artery segment IVUS-VH at the exact same site as that of baseline using similar projections and side branch and/or calcification reference **(Figure 5)** using the technique published by our group[29]. A core laboratory consisting of two experienced cardiologists blinded to the study treatment allocation will review baseline and follow-up coronary arteriograms and IVUS-VH of each patient in the study. These two cardiologists will be independent and have no direct relationship to the study team. If any disagreement occurs between these two cardiologists regarding the angiogram or IVUS-VH interpretation, a third cardiologist will be referred to review the data and break the tie. After visual evaluation, quantitative analysis (QCA) will be performed using a standard automated edge-detection method. Angiographic restenosis is defined as ≥50% reduction in minimum luminal diameter (MLD) of the intervened segment during index PCI.

Figure 8: PREMIER study timelines



Atheroma volumes within a ≥ 20 mm long segment of a target coronary artery segment will be compared to assess change in total atheroma volume and %NC volume. Vessel and lumen area will be calculated for each frame, using previously published methods[18]. Atheroma burden will be calculated as [(EEM area – Lumen area)/EEM area] or expressed as a percent change [(EEM area – Lumen area)/EEM area) x 100][18].VH analysis will be performed for each segment, and the area of each plaque constituent (fibrous, fibrofatty, calcific, and necrotic core) will be determined in an automated fashion using Volcano S5 software version 2.2.3.2236 (Volcano Corp., Rancho Cordova, CA)[32] **(Figure 9)**. Follow-up cardiac catheterization and IVUS-VH analysis as described above will be performed at 90 days from index PCI. This strategy will allow capture of any early change in coronary atheroma volume as a result of the most intensive LDL reduction ever achieved in ACS patients. All follow-up IVUS-VH analyses will be compared to baseline IVUS-VH obtained during the index cardiac catheterization. A more long-term (1 or 2 year) IVUS-VH follow-up is not expected to demonstrate the treatment effect instituted immediately after ACS and has been well described with Nissen et al. with aggressive long-term LDL lowering[18]. Acute changes in coronary atheroma have traditionally been studied by short-term IVUS assessments[20,21].

If a patient has a clinically indicated catheterization within two months of the scheduled study procedure, this would be considered as the follow-up procedure and the operator will be encouraged to procure all study-related data.

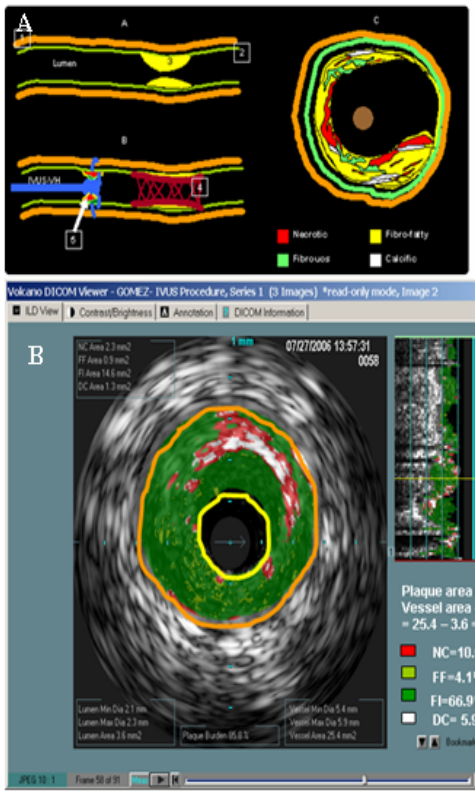
Peripheral blood sampling for EPC quantification will be performed using cell culture only at VA North Texas center in Dallas. This will ensure an optimal number (at least 50) uniformly standardized and cost-effective EPC cell culture assays in this multi-center study. A limited number (n=25) of FACS EPC analysis will be performed to correlate cell culture and FACS levels of circulating EPC. Such a correlation would be important to demonstrate if future large-scale use of this treatment strategy of EPC mobilization is to be implemented with the rapid and less cumbersome FACS instead of the established standard of EPC cell culture technique. Approximately 10ml of whole blood is needed for either assay. Cell culture assay based upon our published methodology[11] will be performed ≤48 hours of collection. EPC flow cytometry will be performed using standard methodology[34]. All EPC sampling schedules are indicated in **Figure 8** and in **Table 1,** along with all other scheduled clinical and laboratory tests. All EPC analyses will be compared to baseline pre-PCI levels. **Table 1** summarizes all planned follow-up visits and tests.

Follow-up tests like fasting lipid (FL) will be performed immediately following LDL-apheresis in ILLT device group at about 12-24 hours post-PCI procedure. The same fasting lipid profile test for SMT control group will be done at 24 hours post-PCI procedure or prior to hospital discharge, whichever comes earlier. A post-PCI 30-day visit will include a complete blood count (CBC) and basic metabolic profile (BMP) with LFT. The 30-day post-PCI CBC, BMP and LFT are clinically indicated tests. A 30- and 90-day FL in both treatment groups, along with EPC assay and an additional 60 day phone follow-up, will ensure strict lipid management and close follow-up for clinical

events, and prevent any loss of recruited study subjects. After the 90-day follow-up cardiac catheterization and IVUS-VH analysis, all study subjects will return in 30 days for a 4-month study visit with CBC, BMP, LFT and FL. After an additional 60 days, or at 6 months from index PCI, all patients will return for a study completion visit and a FL assay. Any MACE will also be collected via searching the Austin database of VA hospital utilization by Hines CSPCC staff. This dual MACE assessment will help avoid missing out on any participating site MACE.

All adverse event, serious adverse event and MACE assessments during follow-up will be performed during patient interviews or history and physical assessments and phone interviews, as indicated in Table 1.

Figure 9: Schematic (A) and on-line in vivo (B) IVUS-VH coronary analysis



A: Coronary vessel stenosis pre-stent; B: Coronary vessel IVUS after critical lesion stenting; C: Region of interest IVUS-VH cross-sectional image reconstruction. 1-External elastic lamina, 2-Intima, 3-Atherosclerotic plaque, 4-Stent and 5-IVUS-VH defined TCFA with > 10% NC at region of interest or ROI (blue hatched line).

B: IVUS-VH longitudinal and the corresponding transverse cross-sectional image, highlighted by the yellow line are displayed on to the right and left of the image screen respectively, during a live recording in our catheterization laboratory. Yellow line demarcates the central vessel lumen (black) with a catheter artifact (blue arrow); Orange line demarcates the external elastic lamina of the vessel. Atherosclerotic plaque components are color coded. The area between the yellow and orange lines, outline the intra-coronary atherosclerotic plaque burden.

Table 1: Follow-up study visits, clinical and laboratory testing schedule

Visits (days)*	H&P	EKG	Card. cath./ IVUS-VH	FL	BMP + LFT	CBC	EPC	Phone Call
Screening/Recruitment	+	+		+	+	+		
Pre-PCI							+	
Index PCI			+					
Post-PCI		+		+	+	+	+	
Hospital Discharge following index PCI for ACS								
30-day f/u visit	+	+		+	+	+	+	
60-day f/u visit								+
90-day f/u IVUS-VH	+	+	+	+	+	+	+	
4-month f/u visit	+	+		+	+	+		
6-month f/u visit	+	+		+	+	+		

*These tests will be performed on all patients in study with "+" indicated when the tests are performed. All follow-up visits calculated from the day of study treatment randomization (dark gray line). Follow-up visits to be scheduled within ± 7 days of that time point in study. 90-day follow-up only to be scheduled ± 15 days of the 90-day time point.
H&P=history and physical; EKG=electrocardiogram; Card. cath./IVUS-VH=cardiac catheterization/PCI/intravascular ultrasound with virtual histology; FL=fasting lipids; BMP=basic metabolic profile; LFT=liver function test;
CBC=complete blood count; EPC=endothelial progenitor cell; PCI=percutaneous coronary intervention;
ACS=acute coronary syndrome; f/u=follow-up.

4(f) Adverse Event (AE) and Serious Adverse Event (SAE) Assessments and Reporting

4(f)(i). Role of the Local Site Investigator in Adverse Event Monitoring: The local Site Investigator will be responsible for following adverse event reporting requirements:

(1). Reviewing the accuracy and completeness of all adverse events reported
(2). Complying with study policies as well as local IRB policies for reporting serious adverse events or problems involving risks to subjects or others in VA research.

(3). Reporting to the IRB any safety issues reported to the site by the Sponsor (Study Chair), and
(4). Closely monitoring of study participants for any new Adverse Events (AE), Unanticipated Adverse Device Effects (UADE), or Serious Adverse Events (SAE).

4(f)(ii). Definitions:

An Adverse Event (AE) is defined as "any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and does not necessarily have a causal relationship with this treatment." An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the study intervention, whether or not related to the study intervention.

A Serious Adverse Event (SAE) is one that results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of an existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require a medical or surgical intervention to prevent one of the outcomes listed in this definition.

An Unanticipated Adverse Device Effect (UADE) means any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

All AE/SAE/UADE with a reasonable causal relationship to the investigative treatment should be considered at least "possibly related." A definite relationship does not need to be established. For the purpose of this trial potential investigative-treatment-related AE/SAE/UADE can be classified into three categories:

(1). Related to LDL-apheresis: hypotension (most common, <1% of treatments in US), nausea/vomiting, flushing, chest pain, fainting, lightheadedness, anemia, abdominal discomfort, numbness/tingling, tachycardia, headache, shortness of breath, hemolysis, bradycardia, itching/hives, bleeding due to the use of unfractionated heparin or low platelet count (thrombocytopenia), low Vitamin E level, temporary decrease in blood protein level, vertigo (dizziness, unsteadiness) and excess sweating.
(2). Related to statin use: muscle pain, generalized body pains, nausea/vomiting, abnormal liver function test (≥ 3 times above the upper limit of the normal laboratory reference values of the study institution), and rhabdomyolysis. Also, on February 28,

2012, the FDA notified clinicians of the following possible effects of statin use: cognitive (memory loss or confusion), which usually reverses after stopping the statin; and increased blood sugar levels (hyperglycemia) that could lead to a diagnosis of type 2 diabetes mellitus. Specifically regarding Lovastatin (Mevacor©), the FDA said that statin in combination with some other medications—including HIV protease inhibitors (e.g. tipranavir plus ritonavir and lopinavir plus ritonavir) and hepatitis C protease inhibitors (e.g. telapravir), and drugs used to treat certain bacterial (e.g. erythromycin, clarithromycin) and fungal infections (e.g. fluconazole, itraconazole, posaconazole, and voriconazole)—can increase the risk of myopathy and rhabdomyolysis. The FDA said serious liver injury due to statin use is rare.
(3). Related to follow-up cardiac catheterization and IVUS-VH: The risk of follow-up study-related cardiac catheterization and IVUS-VH include bleeding, groin pain, arrhythmia, hypotension, pericardial effusion, blood clots, infection, allergic reaction to the dye, perforated blood vessel, air embolism, myocardial infarction, stroke, renal failure, and death. Local groin discomfort and minor access site hematoma (<3 cm in diameter) are most common. Death is rare (<0.04%), and the risk of MI and stroke is <0.5%.

(The examples in these categories are not exhaustive, and investigators should not feel limited by these examples in designating an event “related” or “possibly related” if other AE/SAE/UADE occurs that in their opinion could be potentially related to the investigative treatment.)

4(f)(iv). AE and SAE Monitoring and Reporting: AE/SAE/UADE will be monitored at the study sites throughout the whole period of the study at each clinic visit and telephone contact, beginning as soon as a study participant signs the Informed Consent and continuing through end-of-study for each participant. All AE/SAE/UADE including both those related to the study intervention and those not related to the intervention, will be collected and recorded on the appropriate case report forms (CRF). For the purpose of safety monitoring, the study intervention is defined as the use of 1) statins with or without the addition of LDL-apheresis, 2) LDL-apheresis, and 3) follow-up cardiac catheterization and IVUS-VH as described in this protocol.

All SAEs require expedited reporting. The SAE report form should be completed and faxed to CSPCC/CSPCRPCC/Study Chair Offices within 48 clock-hours of initial awareness of the event. The CSPCRPCC AE Pharmacist will be responsible for evaluating all serious adverse events for patient safety concerns. In addition, a Clinical Events Committee (CEC) will be formed to adjudicate adverse events in the study. The committee involves a physician from CSPCC, a Q.A./safety monitor from CSPCC, and the AE Pharmacist from CSPCRPCC. The committee is independent from the Study Chair and the Site Investigators.

Serious adverse events that are related to the investigative treatment and unexpected will be reported to the FDA, CSP Director, and Site Investigators after review by the Study Chair, the CSPCRPCC Director and the CSPCC Director. Site investigators should follow local policy to inform their IRB. The Hines CSPCC will generate tabulations of all AE/SAE/UADE for the Data Monitoring Committee annually

or on a more frequent schedule if requested by the Committee. All participants presenting to the VA hospital will be provided treatment for all SAEs and UADEs.

While participants have the right to withdraw from the study at any time, they will continue to be monitored, via passive follow-up, for SAEs that occur within 30 days of leaving the study. All unresolved SAEs must be followed up at least every 30 days until resolved or when no further change is expected. All SAEs are followed until no changes are expected or follow-up ends on the last study participant, regardless of the participant's status in the trial.

4(g) Biostatistical Considerations

4(g)(i). Expected Treatment Effect: There is only one study which examined the treatment of medication only (20mg Pravastatin or 10mg Simvastatin) vs. LDL-apheresis with statin medication on coronary plaque regression in familial hypercholesterolemia and published in 2002 Journal of American College of Cardiology[31]. The trial reported the net change of plaque area from baseline to one year follow-up. The changes were -0.69±2.08mm^2 in the LDL-apheresis group vs. 0.88±1.75mm^2 in the medication only group. Two studies, which have primarily mobilized coronary atheroma LDL using HDL or its apoprotein, have shown similar magnitude of atheroma regression at 6-10 weeks, without directly lowering LDL levels. Given the expected 85% reduction in LDL after LDL-apheresis, we expected to observe a similar treatment effect for the primary effectiveness outcome of this study at 90 days.

4(g)(ii). Sample Size/Power/Level of Significance: To maintain 90% power to detect a Cohen's D effect size of 0.65 (-0.38 ± 2.08 mm^2 in the LDL-A group vs. 0.88 ± 1.75 mm^2 in the medication only group) with the randomization scheme of 1:1, $\alpha = 0.05$, and a 20% drop-out rate, the pivotal study needs a sample size of 128.

4(g)(iii). Study Duration/Number of Sites: Four sites are considered for the purpose of generalizability and improved enrollment. Based on the enrollment rate in stage 1 of this trial, sites would expect to randomize 18 patients per site per year with an average rate of 1.5 patients per site per month. The average annual PCI volume during FY 2012 – 2013 reported by VA Cardiovascular Assessment and the data from CSP#571 currently enrolled at these 4 sites indicate that they will be able to meet or exceed the target of 18 participants per year. The percentage of patients with PCI who need to be randomized ranges from 3.5% to 5.0% across the four sites based on these reported annual PCI volume. About 30% of eligible subjects who meet the 3 major inclusion/exclusion criteria (ACS, LDL, and eGFR) need to be randomized in order to meet the accrual target. Dallas and Oklahoma City sites have ongoing LDL-apheresis capability and equipment on-site at the VA campus, and the Nashville and Denver sites have approved LDL-apheresis sites supported by device manufacturer (Kaneka) that will expedite the induction of these sites into the study.

A total of 22 months is needed for 4 sites to randomize the total sample size of 128 in this second stage. The whole study duration for the second stage is expected to

be 46 months including 6 months of start-up, 22 months of enrollment, 6 months of follow-up, and 12 months of database search, study closure, and final data analysis. Upon approval, it is expected that the Dallas and Oklahoma City sites will begin recruitment almost immediately, followed by Nashville and Denver sites.

Estimated Enrollment for CCTA #0002 Phase II							
Site	PCI Volume as Reported by VA Cardiovascular Assessment, Reporting, and Tracking System for Cath Labs (CART-CL)		Annual ACS Indication, FY2014*	LDL > 70 mg/dL AND eGFR > 45 ml/min, FY2014*	Annual Eligible Patients	Monthly Eligible Patients	Monthly Accrual Target
	FY2013	FY2014					
Dallas	350	419	158	94	94	7.8	1.5
Oklahoma City	402	482	258	138	138	11.5	1.5
Nashville	250	313	173	110	110	9.2	1.5
Denver	132	210	115	58	58	4.8	1.5

*Eligibility criteria.

4(g)(iv). Statistical Analysis of Primary Effectiveness Endpoint:

The primary effectiveness outcome will be analyzed with intent-to-treat (ITT) approach by including all randomized patients regardless of crossover or drop-out. The change in total atheroma volume within a ≥ 20 mm long segment of the target coronary artery from baseline to 90 days will be analyzed using the two-sample t-test. We will define their change scores to be 0 if they do not have a 90-day atheroma volume measurement. Analysis of Covariance (ANCOVA) will be performed to evaluate the treatment effect on primary effectiveness outcome adjusting for age, pre-existing CAD, pre-existing diabetes, and baseline LDL levels.

4(g)(v). Statistical Analysis of Secondary Effectiveness Endpoints:

The intent-to-treat principle will also be applied to all the secondary effectiveness outcomes analyses.

(1). The change of %NC component of atheroma from baseline to 90 days will be analyzed using the two-sample t-test. Similarly, the change scores for those who miss the 90 days follow up assessment will be set to zero. Analysis of Covariance (ANCOVA) will be performed to evaluate the treatment effect on primary effectiveness outcome adjusting for age, pre-existing CAD, pre-existing diabetes, and baseline LDL levels.

(2). The EPC-CFU/ml of peripheral blood assessed at various time points (pre-PCI, post-PCI, 30 days of follow-up, and 90 days of follow-up) will be analyzed via Mixed Linear models with random intercepts. The treatment effect, time effect and their interaction will be considered with or without

adjusting for age, pre-existing CAD, pre-existing diabetes, baseline LDL levels, and other relevant covariates such as ACS, peripheral vascular disease, and chronic kidney disease. The statistical test of interest is the time by treatment interaction.

(3). Contrasts will be used in the Mixed Linear models to compare the differences among any specifically interested time points.

4(g)(vi). Statistical Analysis of Safety Endpoints and Complications: The primary safety endpoints, the secondary safety endpoints, and the incidence of major adverse cardiovascular endpoints (MACE) including death, myocardial infarction, coronary revascularization, and stroke will be analyzed in two ways. For each type of event, the percentage of people who experience the event at least once will be compared using the chi-square test. Logistic regression models will be performed to adjust for the covariates such as age, pre-existing CAD, and pre-existing diabetes. In addition, a similar analysis will be done for all AE and SAE that are possibly or probably attributable to the study intervention.

An event-based analysis will also be done since adverse events can be recurrent. In this analysis, a non-parametric method called the mean cumulative function (MCF) [35,36] will be used as an alternative to the above crude incidence rate analyses. The overall safety profiles as well as the safety profiles in specific subgroups over the whole study period will be compared for the two randomization groups including the times of event recurrence and censoring mechanisms.

Time to event (survival analysis) will also compare the time to first MACE between treatment groups. A second survival analysis will be performed to identify risk predictors and to evaluate whether the observed treatment effect is modified by adjusting for covariates such as pre-existing CAD and pre-existing diabetes.

4(g)(vii). Interim Monitoring: Hines CSPCC will produce a progress report every six months for review by Data Monitoring Committee. The report will include figures for patient accrual, tables for baseline characteristics, site performance, data quality, treatment compliance, and safety issues. Group sequential methods will be used to specify the α-levels in order to maintain the overall significance level at $\alpha = 0.05$ for the primary effectiveness hypothesis test. The sequential analyses will use O'Brien-Fleming boundaries with an overall $\alpha = 0.05$ for significance and 90% for power. EAST software was used to obtain the interim monitoring rule and the adjusted sample size. The look will be around 50% of the target sample size (about 64 patients) completing the 90-day follow-up visit as DMC recommended at its first meeting on September 25, 2009. The sample size remains at 128 to account for this extra interim look by assuming the groups mean difference of -1.26 and the pooled standard deviation of 1.922.

The interim look will cost 0.003 for α. If the critical value is greater than 2.9626 or less than -2.9626, the study will be stopped to reject the null hypothesis. However, if the critical value is between -2.9626 and 2.9626, the study will continue until the target sample size is met for final analysis.

4(g)(viii). Subgroup Analyses: Since acute coronary syndrome (ACS) is manifested as either unstable angina or non-ST-elevation myocardial infarction, both

safety and effectiveness outcomes will be analyzed for LDL-apheresis treatment effects in unstable angina subgroup and non-ST-elevation MI subgroup as well as the total ACS patients. In addition, separate analyses will be performed across 4 participating sites for each treatment group in order to evaluate the site effect and confirm the appropriateness of pooling the data for primary endpoint.

4(g)(ix). Sensitivity Analysis for Missing Data: In addition to the above mentioned ITT principle for primary and secondary effectiveness outcomes in which the change scores will be set to zero if the follow-up assessment is missing, other approaches will be applied in order to assess the robustness of the study results to assumptions about the missing data. For outcomes about atheroma volume and % of NC component of atheroma which will be measured only at 2 time points, the sensitivity analysis approaches include: 1) performing analysis only on complete data, 2) applying the worst case scenario (set the lowest score at baseline and the highest score at 90-day), and 3) generating imputes for missing data with multiple imputation. For outcome about EPC-CFU/ml of peripheral blood which will be repeatedly measured at four time points, the sensitivity analysis approaches include: 1) performing analysis only on complete data, and 2) using the mixed model for repeated measures.

All tests will be two-sided. The critical level for all the primary and secondary outcomes will be 0.05. No adjustments will be made for multiple secondary endpoints.

4(h) Quality Assurance, Oversight and Study Regulation

4(h)(i) Training to assure accuracy, precision and validity of the data: Toward the end of the six-month start-up period, training of study personnel will take place during a two-day kick-off meeting. All participating investigators, site research coordinators/raters, and Hines CSPCC personnel will be in attendance. Prior to the meeting, case report forms will be finalized and a detailed manual of study operations will be written and circulated. This manual will serve as the training manual for the meeting and as a reference document following the meeting. The PI's office and the data and pharmacy coordinating centers will provide the training. The general training will include the study treatment, patient screening and consent, baseline evaluation, follow-up procedures, and proper entry and maintenance of data. Cyber security awareness training and privacy training (VHA Privacy Policy, Protection of Human Research Subjects, and Good Clinical Practices) are required annually of all VA and VA-WOC employees.

4(h)(ii). Data Management and Security

4(h)(ii)(1). Data Collection: An operations manual and case report forms will be developed jointly with Hines CSPCC. The site study coordinators will collect all data. After a patient consents to participate in the study, the site coordinator will create a patient casebook, which will contain the consent forms, all relevant source documents, and any other information pertinent to the study. The completed case report forms will

be assembled and faxed to Hines CSPCC for further handling on a daily basis. These original completed paper forms will be kept in the Site Investigator's study files as source document.

4(h)(ii)(2). Data Quality Assurance: DataFax, a clinical trial data management system (by Clinical DataFax Systems, Inc.), will be used for data collection and management. The study coordinators from the sites and the Independent Assessors will complete case report forms (CRFs) in paper and fax them directly to the DataFax computer server, where data images of the CRFs are stored as files. The system uses an optical character recognition (OCR) paradigm to automatically process and store the information from the image as data into the study database. The original fax image is also stored. Data management staff at Hines CSPCC will review each CRF by comparing the faxed image with the OCR data and ensure that the two match. Data management staff at Hines CSPCC will also review CRFs for protocol adherence, data consistency, and add data queries to items that fail these checks. Extensive data checks, including missing values, out-of range entries, and consistency between variables, both within and across forms, will be performed manually and programmatically. On a regular basis, data management staff will produce site-specific Quality Control reports that list all unresolved data queries. Data management staff will make the reports available to each site and work with the study coordinators and the Independent Assessors to help them resolve queries. Queries will be resolved when the appropriate corrections to the CRF are made and data resent, or when an explanation is provided that allows for data management staff to resolve the query. All corrections and changes to the data will be reviewed by data management staff. In addition to the Quality Control report, Hines CSPCC may generate and distribute targeted data edit reports on an as-needed basis. The Hines CSPCC will monitor completeness and timeliness of the data discrepancy resolution made by study coordinators at participating sites. The Study Chair, the study coordinators and Independent Assessors will receive periodic reports regarding the quality and quantity of data submitted to the Hines CSPCC. Other quality control measures include periodic reports containing participant recruitment information and relevant medical data for review by the Study Chair. The Hines CSPCC will also prepare summary reports for the Study Chair, the Data Monitoring Committee, and other monitoring groups of the data to track progress, and conduct final analyses of the study data. Study reports will be generated using DataFax, SAS, and other tools (e.g., Microsoft Excel and Access). SAS and other statistical software packages will be used to conduct data analysis for the study. The Hines CSPCC is using SAS Version 9.3 in 2014 and will upgrade to newer versions once they are purchased and validated.

4(h)(ii)(3) Data Confidentiality and Data Security: Handling and storage of study data will adhere to current VA policies. The analytical database will not contain information that can directly identify the study participant (such as name, address, etc.); however it will not be a completely de-identified database since age and study visit dates will be collected. The Hines CSPCC requires that a copy of the signed consent form and a participant contact sheet be on file at the Hines CSPCC. The consent form is required by CSP policy in order for the coordinating center to independently certify that

all study participants have been consented. Because the Hines CSPCC is the final data repository for the study, participant contact sheets are collected in the event study participants need to be contacted (such as for safety notices) after study sites have completed the study. Consent forms and participant contact sheets are stored separately from study data. All paper-based records, including source documents and paper case report forms, will be kept in locked file cabinets at the participating sites and Hines CSPCC. The servers housing the study databases will be located at a secure VA facility and housed behind the VA firewall on VA-owned and VA-maintained servers. The system will be monitored to ensure that all applicable VA regulations and directives are strictly followed. Access to the study data is restricted by the Hines CSPCC to properly credentialed research staff who have completed required VA security trainings. Only CSP-approved individuals (such as: staff at the study sites, CSPCC, and CSP Clinical Research Pharmacy Coordinating Center [CSPCRPCC]) will have access to the personal health information (PHI) of study participants.

4(h)(ii)(4). Site Performance: Hines CSPCC evaluates recruitment and retention performance monthly. Other performance problems such as protocol deviations, poor data quality, missing or overdue data, and reasons for withdrawal from study will also be tracked. A monthly study conference call for study personnel including Hines CSPCC, CSPCRPCC, Chair's office, and all sites will be held to review study recruitment, data quality, and protocol adherence. Study sites will be put on probation for poor performance, including under-recruitment. Typically the probationary period is three months, at which time the site may be taken off probation, have probation continued, or have funding stopped, depending on its performance during the probationary period. The Hines CSPCC Director is authorized to make these decisions.

4(h)(iii). Study Monitoring: The groups charged with centrally monitoring the various aspects of the study will be the Executive Committee (EC) and Data Monitoring Committee (DMC). Both committees meet at study start-up and annually thereafter. The EC will also have quarterly conference calls. The DMC may elect to meet more frequently if it deems it necessary.

The Executive Committee is the management and decision-making group for the operational aspects of the study. One of its major responsibilities is to monitor the performance of the participating medical centers. The EC considers the need for protocol modifications. The Executive Committee also reviews and approves all manuscripts and abstracts emanating from the study. Typically the EC is composed of the original study Planning Committee. It is chaired by the Study Chair.

The Study Group, which consists of all participating investigators and site clinical research personnel, will meet annually to discuss the progress of the study and any problems encountered during the conduct of the trial. Study personnel will adhere to Office of Research & Development (ORD) policy on human subjects' protections by fully completing approved Good Clinical Practices (GCP) training per VHA Handbook 1200.05. Completed training certificates will be provided by site personnel to the Hines CSPCC to maintain on file throughout the study. SMART (Site Monitoring, Auditing, and Resource Team), a division of the CSP Clinical Research Pharmacy Coordinating Center (CSPCRPCC), may conduct a full audit of participating sites if requested by any

of the monitoring bodies. The study will be monitored utilizing centralized data and statistical monitoring methods and remote-based source verification; which will be based on risk assessment and management strategies.

The Data Monitoring Committee will review the progress of the study, including patient intake, completeness of follow-up, data quality, protocol deviations, and safety. The DMC may also choose to implement a formal interim monitoring rule to monitor efficacy. The DMC will review any protocol modifications recommended by the EC. The DMC will establish criteria for study termination and make recommendations to the Director, CSR&D, through the Director, Hines CSPCC, as to whether the study should continue, be put on probation, or be terminated. Interim unblinded progress reports will be provided to the DMC by the study biostatistician. This study has been assigned to a central DMC organized and managed by VA Clinical Sciences Research and Development (CSR&D). The membership of DMC is approved by the VA CSR&D Director and has no direct relationship to any facility, study or investigator. The DMC has about 10 members, in which three members including one interventional cardiologist, one critical care physician, and one biostatistician were assigned to review this study.

The Study Group, which consists of all participating investigators and study personnel at Hines CSPCC and Albuquerque CSPCRPCC, will meet annually to discuss the progress of the study and any problems encountered during the conduct of the trial. Finally, the study protocol and progress must be reviewed annually by the local R&D Committee and IRB at each site in order for that site to participate in the study.

4(h)(iv). Study Performance Benchmarks: Study benchmark dates will be determined upon successful funding of the study. However, the following timelines will serve as a reference:

(1). Enrollment of four VA sites: Dallas VA, and 3 other VA sites
(2). Study site IRB submission and approval: four months
(3). Study sample transportation and storage arrangements and contract: two months
(4). Recruitment of ACS patients/month/site: one and a half
(5). Study sites recruitment/year: 72
(6). Subject follow-up and follow-up study blood sampling: 80%
(7). Lost to follow-up: <20%

4(i) Human Subjects:

1. Human subject involvement and characteristics: As the study proposes to enroll ACS patients, strict enrollment criteria will be used to select eligible patients.

2. Sources of material: The source of material will be information obtained during hospitalization, procedure logs, and laboratory values and tests. The laboratory values and tests will include research laboratory and patient relevant hospital laboratory data. LDL-apheresis data will be collected by the apheresis center nurse on paper forms. Patients' names will not be divulged, and all data will be coded in the study records. The investigators will only use information gained

during the study for the evaluation of study results. The copies of signed informed consents will be kept on file by the site investigators, and no patient names or other identifying data will be used in future publications. All files will be secured in a locked filing cabinet in a locked office.

3. Informed consent: The site investigators or the site study coordinators will interview and explain the details of the study to all potential subjects, who will be subsequently asked to sign an IRB-approved informed consent form prior to any study procedure.

4. Provisions for managing adverse reactions: Only trained and accredited personnel will be performing PCI, IVUS, LDL-apheresis, peripheral blood sampling, and laboratory handling of specimens. Cardiac catheterization will be performed using standard clinical protocol, and all patients will be observed closely in hospital after the procedure and discharged when clinically indicated. Consented patients with a complicated PCI procedure will be excluded from participating in the study, but will be monitored for AEs/SAEs, and the safety data will be reported to the study safety and oversight committees. However, randomized patients with post-PCI complications will still be followed unless otherwise terminated from the study, performed as many assessments as possible, and included in the final analyses. Intensive patient follow-up protocol has already been described in **Table 1**. Patients will undergo LDL-apheresis using peripheral IV access exclusively. No more than two patients will be enrolled and will undergo LDL-apheresis on a given day at a center. The study will be terminated if recommended by the oversight committee at any point if potential harm to the patients is demonstrated.

5. Potential benefit to subjects and others: All patients will be treated and followed following an ACS and will receive more intensive pharmacologic hyperlipidemia management which has traditionally resulted in improved patient outcomes. Patients will also be reimbursed $50.00 for participation in the study, $50.00 for the 90-day follow-up visit, and $40.00 for each of the other follow-up visits to improve compliance and attainment of National Cholesterol Education Program (NCEP) and the latest ACC/AHA Lipid Lowering Therapy guidelines. The information obtained from this study could certainly develop as a viable strategy for our veterans. Successful completion of this study with acquired necessary experience may lead to development of this treatment strategy at one of the largest VAMCs in the nation.

6. Importance of the knowledge gained: The results of this study, apart from providing VP stabilization information from intensive LDL lowering, will principally advance the field of coronary atherosclerosis and VP healing.

4(j) Publication Policies and Planned Manuscripts

The study findings will be presented at national meetings and published in peer-reviewed medical journals. Before the publication phase, the study's objectives and other related facts will be presented for informational (not recruitment) purposes on the Cooperative Studies Program website at www.research.va.gov/programs/csp/ccta0002. Only general information will be provided without any participant-specific references. The Executive Committee will approve all topics for presentation or publication. It will review and approve all manuscripts and abstracts prior to submission. The Director, Hines CSPCC, must also approve every manuscript and abstract prior to publication.

4(k) Resources

All work including LDL-apheresis will be conducted on the premises of four study sites except that the EPC cell culture analysis will be done only at the Dallas VAMC:

1. Cardiac catheterization and PCI/IVUS-VH will be performed in the cardiac catheterization laboratory of the Dallas VAMC and other VA sites.

2. Laboratory analysis of EPC by cell culture and flow cytometry will take place in the research laboratory of the PI at Dallas VAMC. The PI will be utilizing a dedicated laboratory space, which currently includes 850 sq ft of laboratory space, 200 sq ft of office space, and shared 400 sq ft dedicated to cell culture facility. The same building houses common-use instruments such as ultracentrifuges; inverted dual laser confocal microscope (Zeiss); inverted microscope (Nikon) with epifluorescence, Nomarski optics, and digital image acquisition (Metamorph); flow cytometer (BD FacsCaliber); cryotome and microtome, and molecular imager (BioRad). All equipment for performance of the project is available to the PI.

3. Single LDL-apheresis in all study patients enrolled will take place at the intensive care and inpatient hemodialysis rooms. Dr. Banerjee has attended several apheresis sessions conducted at UT Southwestern Medical Center in Dallas and during the phase 1 of the study to obtain first hand experience and didactic education. A specialized LDL-apheresis nurse coordinator will be recruited and perform the apheresis procedure under the supervision of an apheresis expert identified as site co-investigator. Similar to Dallas, arrangement regarding the apheresis expert will be done at other participating sites.

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