Effect of Vascepa on Progression of Coronary Atherosclerosis in Persons with Elevated Triglycerides on Statin Therapy

Investigators
Matthew Budoff, MD

Co-Investigators
J. Brent Muhlestein, MD
Viet Le, PA
Heidi T. May, PhD, MSPH
John R. Nelson, MD
Sion Roy, MD

Corresponding Investigator:
Matthew Budoff, MD
1124 W Carson Street
Torrance, CA 90502
(310) 222-4107
Budoff@ucla.edu

Sponsoring Institutions
Los Angeles Biomedical Research Institute
Intermountain Heart Institute, Intermountain Medical Center
California Cardiovascular Institute

Research funding
Amarin

Protocol: Version 3, February 13, 2017

ClinicalTrials.gov Identifier: NCT02926027

BACKGROUND

Residual cardiovascular (CV) risk remains in dyslipidemic patients despite intensive statin therapy, underscoring the need for additional intervention. Eicosapentaenoic acid (EPA), an omega-3 polyunsaturated fatty acid, is incorporated into membrane phospholipids and atherosclerotic plaques and exerts beneficial effects on the pathophysiologic cascade from onset of plaque formation through rupture. Specific salutary actions have been reported relating to endothelial function, oxidative stress, foam cell formation, inflammation, plaque formation/progression, platelet aggregation, thrombus formation, and plaque rupture. EPA also improves atherogenic dyslipidemia characterized by reduction of triglycerides without raising low-density lipoprotein cholesterol. All of this data supports the biologic plausibility of EPA as an anti-atherosclerotic agent (1). In the Study on Prevention of Coronary Atherosclerosis Intervention with Marine Omega-3 Fatty Acids (SCIMO) data has shown that omega-3 PUFA (both DHA and EPA) slow progression of atherosclerosis in coronary arteries [2]. However, this data used invasive angiography. There is only limited data regarding the effects of EPA without DHA added to statin versus statin alone therapy in reducing coronary plaque volume. Utilizing integrated backscatter intravascular ultrasound (IB-IVUS), 1.8 grams/day of EPA added to standard statin therapy, unlike statin therapy alone, reduced plaque volume and inflammatory cytokines over 6 months of follow-up (3). Recently, a second study using IB-IVUS showed reduction in both plaque and lipid volume over 6 to 8 months in patients who were treated with 1.8 grams/day of EPA and high dose pitavastatin (4mg/day) unlike high dose pitavastatin alone (4). Data regarding the effects of EPA on plaque as measured by multidetector computed tomography (MDCT) are even more limited, with an abstract reporting that MDCT showed that patients randomly assigned to EPA, unlike ezetimibe, reduced plaque lipid content at 1 year (5).Furthermore, a case report described a patient with plaque regression under the influence of EPA (6).

MDCT allows for a robust study of atherosclerosis at a nominal cost compared to intravascular ultrasound or invasive angiography to demonstrate that Vascepa has a positive effect on atherosclerosis, to complement the outcome study ongoing. This becomes a critical question among cardiologists, endocrinologists and primary care physicians, who will want to see event reduction and plaque reduction to feel compelled to use this important intervention on their high triglyceride patients. MDCT is now used in numerous ongoing studies evaluating such therapies as testosterone, statins, hormone replacement, garlic, anti-inflammatories and anti-diabetic agents. The ease of use, low cost and minimal invasiveness makes this much more practical and desirable as a study tool for atherosclerosis than either intravascular ultrasound or carotid intimal media thickness. A small multicenter study can determine with high confidence the ability of Vascepa to modify plaque in persons with high triglycerides.

Multiple studies have been published regarding the use of MDCT angiography on plaque over time. Initial study sample size is estimated based on MDCTA data showing 24±13% reduction in coronary artery plaque volume with statin therapy (6). Other CCTA studies performed by our group also demonstrate rapid plaque changes over time, and significant differences between groups. In a 6 month follow-up study of patients with ACS treated with a 5 lipo-oxygenase inhibitor or placebo, cardiac computed tomography angiography (CCTA) demonstrated a 21% increase in plaque in the placebo treated population (including statins) (7). In a second placebo controlled randomized study, placebo patients (on statins), exhibited an 8.1% change in plaque, significantly greater than those randomized to a garlic preparation (5). In a third study of patients with diabetes, the plaque

progression by CCTA in the entire cohort was 24% per year, a 2.5 fold the rate of change seen in non-diabetic patients (8). We have also demonstrated significant differences in 1 year time between statin treated and non-statin treated cohorts (10).

While clinical utility of Vascepa is well established in the >500 mg/dl triglyceride level group, there remains questions about use in lower TG values. The ability to retard progression or induce regression of atherosclerosis would dramatically drive utilization and clinical confidence in the therapies. Patients and physicians alike are looking for therapies, in addition to statins, to address this very high clinical need. The number of therapies available for triglycerides are plentiful, so differentiating Vascepa in this crowded field will be met with great enthusiasm. More evidence of benefit, along multiple lines, is clearly a mechanism to improve confidence for physicians and patients alike.

The goal of this study is to evaluate whether treatment with Vascepa (4 grams) results in a greater change from baseline in low attenuation plaque than placebo in subjects with elevated triglycerides (200-499 mg/dl).

HYPOTHESIS

Primary Hypothesis: Vascepa (4 grams/day) and statin therapy will reduce progression of low attenuation plaque volume over 9-18 months as measured by serial coronary CT angiography (CTA) as compared to statin alone in patients with triglycerides 200-499 gm/dl. The mechanisms underlying the coronary effect of EPA will include a) reduction in non-calcified coronary atherosclerotic plaque, b) reduction in vulnerability features of non-calcified coronary atherosclerotic plaque.

Secondary Hypothesis: 1) Vascepa will significantly reduce non-calcified coronary plaque and total plaque volume progression as compared to placebo. 2) Decreases in plasma levels of triglycerides, remnant lipoproteins ($VLDL_3$-C + IDL-C), Arachadonic acid (AA), an improvement in critical indices of inflammation, and an increase in the apoA1 to remnant ratio (apolipoprotein A1/[$VLDL_3$-C + IDL-C]), EPA, EPA/AA ratio associated with EPA therapy will be predictive of improvement in non-calcified coronary atherosclerotic plaque burden and/or vulnerability features.

STUDY OBJECTIVES

Primary Objective: Determine progression rates of low attenuation plaque under influence of Vascepa as compared to placebo.

Secondary Objectives: a) To determine the effects of Vascepa on the morphology and composition of non-calcified coronary atherosclerotic plaque (NCP), including the progression of total plaque volume and whether these effects are modulated by lipid changes and markers of inflammation; b) To determine the effects of Vascepa on detailed markers of inflammation (Lp-PLA_2, hsCRP, IL-6), lipids and lipoproteins (ox-LDL, remnants ($VLDL_3$-C + IDL-C), apoliprotein A1 to remnant ratio, EPA, AA, EPA/AA ratio), and traditional CVD risk indices.

METHODS

A randomized double blind trial that compares Vascepa 4 gm/day with placebo among patients with elevated triglycerides (200-499mg/dl). Patients will be educated to maintain a low cholesterol diet through education to patients as well as statin medication compliance.

Study Design

Patients will be randomized 1:1 to Vascepa or placebo to evaluate progression rates of low attenuation plaque volume. At multiple centers, a total of 80 eligible subjects will be enrolled, with a goal of 70 completing the study. Patients will be evaluated for efficacy at an interim evaluation at 9 months by statistician and DSMB and if efficacy is not achieved then patients will be followed for additional 9 months to determine lack of progression of low attenuation plaque volume at 18 months.

Randomization and Stratification

Subjects who meet the eligibility criteria will be randomly assigned to receive Vascepa or matching placebo.

Inclusion and Exclusion Criteria

Inclusion Criteria

* Age 30-85 years
* Elevated triglycerides (fasting value between 200-499 mg/dl) at qualifying or baseline visit
* Subjects must provide written informed consent after the scope and nature of the investigation has been explained to them
* LDL-C ≤115 mg/dL on appropriate statin therapy
* LDL-C ≥40 mg/dL
* Stable diet and exercise, as defined as the same pattern for the previous 4 weeks
* Stable treatment with a statin+/- ezetimibe for at least 4 weeks
* Patients with at least 1 angiographic stenosis with at least 20% narrowing by coronary computed tomography angiography (CTA).
* Willingness to be on birth control for women of childbearing age or established post-menopausal

Exclusion Criteria

* A contraindication to fish or fish oils including: known hypersensitivity to drug or fish
* Any unstable medical, psychiatric or substance abuse disorder that in the opinion of the investigator or principal investigator is likely to affect the subject's ability to complete the study or precludes the subject's participation in the study
* Non-study lipid altering medications or supplements (ie – Niacin, PCSK9, fibrates, bile acid Sequestrants, dietary fish oil supplement capsules, orlistat [OTC (Alli®) as well as Rx (Xenical®)] or other drugs used for weight loss)
* Stable (same daily dose for the last 4 weeks) on medications that can affect lipids (retinoids, hormones, steroids, HIV medications, chemotherapy, thyroid medications)
* BMI > 40
* Bleeding disorder

* Uncontrolled hypertension (SBP≥ 180 mmHg or DBP≥100 mmHg)
* History of known myocardial infarction, stroke or life-threatening arrhythmia within the prior six months
* NYHA Class III- IV heart failure
* History of malignancy within the last 5 years (other than skin cancer) or evidence of active cancer which would require concomitant cancer chemotherapy
* Serum creatinine > 1.4 mg/dl
* Drug or alcohol abuse, or current intake of more than 14 ounces of alcohol per week for men and 10 ounces for women
* Concurrent enrollment in another placebo-controlled trial or within 30 days of finishing another trial
* Partial ileal bypass or known gastrointestinal disease limiting drug absorption
* History of hypertensive encephalopathy or cerebrovascular accident
* Hematological or biochemical values at screening outside the reference ranges considered as clinically significant in the opinion of the investigator or PI
* Pregnancy
* Genetic mutations/polymorphisms having an effect on blood lipids
* History of coronary artery bypass surgery
* Allergy to contrast material
* Allergy to beta-blocker in subjects with resting heart rate >70 bpm

Endpoints

Primary: Rate of change in low attenuation plaque volume as measured by MDCT angiography.

Secondary Endpoints

1. To assess incident plaque rates and quantitative changes in different plaque types among patients randomized to placebo and Vascepa using CTA.
2. To determine the effects of Vascepa on detailed markers of inflammation (Lp-PLA_2, hsCRP, IL-6).
3. To determine the effects of Vascepa on lipids and lipoproteins (ox-LDL, remnants [$VLDL_3$-C + IDL-C], apoliprotein A1 to remnant ratio, EPA, AA, EPA/AA ratio)

Power Calculation

Assuming 80% and an alpha of 0.05, a total of 70 (35 per arm) patients are needed to evaluate the primary outcome of the study (EAST, version 6.3; Cambridge, MA). The primary outcome is the progression rate of low attenuation plaque volume at final assessment compared to baseline assessment. Initial study sample size was estimated based on MDCTA data showing 24±13% reduction in coronary artery plaque volume with moderate dose statin therapy (7). We assume an 8% reduction in plaque volume (1/3 that seen with statin therapy), would be the minimal important change in non-calcified plaque volume. Multiple studies have shown similar progression rates under the influence of different therapies (8-11). Furthermore, ample data has been obtained using low attenuation plaque (LAP) volume in both MDCT and IVUS Studies. In the Niki study (3) Statin +EPA vs Statin alone at 6 months), at lipid volume decreased from 18.5 to 15.0(18.9%); control 17.8 to 19.8-increased 11.2% (difference 30.1%). The CHERRY study, (Pitavastatin +EPA vs Pitavastatin alone for 6-8 months), lipid volume decreased from 39.2 to 34.8 (11%); control 42.7

to 39.3 (8%). Thus, plaque decreased between 11 and 18.9% in two studies in the EPA arms. Furthermore, in the Shintani study of EPA vs ezetimibe, there was a change of 29.2-25.1 (decreased 14%), using the same range of HU that we are using to represent LAP. Based on these 3 trials, an 8% improvement in LAP in the EPA arm of the trial being proposed in this protocol is reasonable.

Assuming an average of 1.7 measurable plaques per study subject, with intra-subject plaque correlation of 0.24 (12) (providing variance inflation factor of 1.06 to account for lack of statistical independence of plaques within same study subject) (13), 70 patients would provide power of 0.80 and a one-sided type I error of 0.05 to detect a 8% difference in plaque volume between the active compared to placebo group. Overall change for each plaque type from baseline will be calculated for each patient. Assuming a 15% dropout rate, a total of 80 (40 per arm) patients would need to be enrolled.

Interim Analysis

Using the Lan-DeMets version of the O'Brien-Fleming group sequential boundaries for a 2-look sequential design (1 interim at 9 months + final analysis), the statistical power to test the primary study endpoint is 80% based on a sample size of 70 patients randomized in a 1:1 allocation ratio and an overall experimental type I error equal to 0.05 using a 1-sided hypothesis test. If a p-value of $\leq$0.006 is achieved at 9 months then the study will terminate because the efficacy boundary will have been achieved.

Statistical Analysis

Baseline examination of the subjects will include the results of their demographics, coronary risk factor, laboratory tests, coronary calcium, as well as coronary plaque volume/composition. These baseline characteristics will be compared between the arms. Baseline information regarding risk factors for atherosclerotic cardiovascular disease (cigarette smoking status, systemic hypertension, family history of premature atherosclerosis, menopausal and hormone replacement status in women, sedentary lifestyle, current medications, chest pain questionnaire and measures of obesity, diabetes medication use) will also be determined. After randomization, participants will evaluated at 3, 9 and 12 months, and if needed 15 months (by phone-only) and 18 months, to assess compliance with medication, and receive an additional supply of medicine. At 9 and 18 (if needed) months, coronary plaque volume/composition will be measured at MDCT by readers blinded to the randomization and clinical activities, who are not aware of study-group assignments. The primary analysis will utilize intention-to-treat principles, with study subjects analyzed by treatment group assigned regardless of study drug adherence. A sensitivity analysis will be performed for those adhering to interventions for over 80% of the duration of the study. Differences in baseline characteristics between groups will use analysis of variance for normally distributed continuous traits and chi-square or Fisher's exact test for categorical variables. For normally distributed continuous outcomes, including percent change in low attenuation plaque, we estimate mean percent group differences using linear mixed models with baseline value, time and treatment group as fixed effects. Least squares means will be estimated from the models. Models evaluating per patient outcomes will include fixed effects. Natural log-transformations will be used for variables with log-normal distributions. For the analyses of the primary, secondary, and exploratory efficacy variables, the statistical modeling assumptions should be examined. If significant departures from normality (p-value <0.01 for the Shapiro-Wilk test) and/or homogeneity of variance are observed, the non-parametric analysis approach will be considered. When parametric and non-parametric results do not corroborate each other, the results from the non-parametric analysis will be used.

Otherwise, the parametric analysis will be reported. Baseline characteristics will be provided as mean and standard deviation or median and interquartile range, changes from baseline are provided with 95% confidence limits. All statistical tests report 1-sided *p*-values for the outcomes. A *p*-value less than 0.048 will be considered significant for the outcomes. All analyses will be performed using SAS for Windows, version 9.3 (SAS Institute, Cary, North Carolina).

CCTA EVALUATIONS

CCTA

As successfully performed in previous NHLBI-funded investigations by the PI, CCTA scans will be read in a blinded fashion at the MESA CT Reading Center (RC). CCTA will be performed using state of the art 64+ MDCT technology. At Intermountain Health, a 64-slice coronary computed tomography (CT) scanner (Aquilion 64, Toshiba America Medical Systems) will be used. At Los Angeles Biomedical research Center, a Revolution 256-detector scanner (GE Medical, Milwaukee, WI). Both sites will pass quality control training under RC supervision and have extensive expertise in performing CCTA. All participants will be hydrated pre and post scan. Nonionic contrast material will be used. The CCTA study will incorporate all dose reduction strategies available, including prospective ECG-triggering and iterative reconstruction. Participant burden for the CCTA is anticipated to be 1 hour.

CCTA Protocol

1. Renal function will be measured within 3 months of the scan to determine eligibility for contrast injection.
2. Preprocedural Medications [beta blockade (only when needed for increased heart rate)]

a. Metoprolol: If the heart rate is >70 beats per minute (BPM), 50 mg of metroprolol will be given orally and if HR remains >70 BPM at the scan acquisition then, intravenous metoprolol 2.5-5 mg every 5 minutes administered to achieve a heart rate between 50-70 BPM as blood pressure tolerates under physician supervision. Participants may receive intravenous diltiazem 10-25 mg IV if contraindicated for β-blockade (reactive airway disease).

b. Nitroglycerin: Unless systolic blood pressure is less than 90 mm Hg supine or known nitrate intolerance, participants will receive 0.4 mg of sublingual NTG one minute before contrast scan initiation to improve epicardial vasodilation, unless the participant took Viagra, Cialis or LeVitra within 24 hours of the study.

Contrast Administration

CCTA will be performed during a 4-5 ml/sec intravenous iodinated infusion. The total contrast dose will not exceed 70 ml. Depending on participant size, 50-70 ml iodinated contrast will be used. The automated bolus tracking feature will be used to judge contrast bolus arrival and optimize image quality. Participant breath hold during scanning will be approximately 10-15 seconds.

CCTA Evaluation

The CT reader will interactively use axial images, multi-planar reconstructions (MPR) and maximum intensity projections to assess the degree of luminal narrowing stenosis in all assessable coronary segments. Standard display settings will be used for the evaluation of the CCTA scans (window width 800 HU; window center 250 HU). Stenotic segments will be defined as minimal (1-25% diameter narrowing); mild (26%-50%); moderate (51-70%) and severe (>70% stenosis). We will specifically report % diameter stenosis (not area), since the spatial resolution of CCTA cannot

achieve quantitative coronary angiography precision. The most narrowed diameter in each segment will be reported even if the plaque is eccentric. Segment stenosis score will also be generated based on the degree of underlying stenotic disease in each segment (0=none, 1=1-25%, 2=26-50%, 3=51-70%, 4=>70%). The summed scores of 15 segments will yield a total score from 0-60.

CAC METHODOLOGY

After arterial trajectories are determined and a phantom-based adjustment applied, candidate calcified plaques will be identified by the software with the criteria that each plaque be composed of at least 4 contiguous voxels with an attenuation level of 130 HU or greater. The readers review each candidate plaque and accept or reject its classification as calcified plaque. To calculate the Agatston score, each accepted lesion is assigned a score by multiplying the lesion volume by a coefficient based on its maximum HU (coefficient of 1 if maximum=130-199, 2 if 200-299, 3 if 300-399, 4 if ≥400). The Agatston score is the sum of the scores across all accepted lesions. We will also derive volume scores, and density measures of each plaque.

Plaque quantification

Plaque volume will be assessed per slice in all affected coronary segments measured by semi-automated quantification software (QAngio, Medis, Netherlands). First, an automatic tree extraction algorithm will be used to obtain all the 3-dimensional centerlines of the coronary tree. Based on these centerlines, straightened MPR volumes are created of all vessels. Next, the lumen border contours and vessel wall borders are assessed using spatial first- and second-derivative gradient filters in longitudinal cross sections. Thereafter lumen and vessel contour are detected in the individual transversal cross-sections perpendicular to the centerlines. This method is insensitive to differences in attenuation values between data sets and independent of window and level settings. Once automated software has completed the vessel trace, an expert reader will manually correct areas of misregistration. The volume of each plaque visualized in at least 2 adjacent slices (slice thickness 0.6 mm) will be determined. For each lesion, minimal lumen diameter will be summed and plaque reported as non-calcified, low attenuation or calcified. The protocol for quantitative plaque assessment has been widely used in numerous previous studies by the PI, including active NHLBI studies: R01 HL095129 (MACS) and 5U01AG030644 (T Trials, PI- Snyder).

Plaque Composition

Plaque Composition is based upon predefined fixed intensity cutoff values of CT attenuation. These are based upon studies by comparing CCTA with virtual histology by IVUS or histological examination in our lab and others. The fixed HU cut-off values that will be used for classifying are: -50 to 50 for low attenuation plaque, 51–130 for non-calcified, 131–350 for fibrotic, and >350 for dense calcium. These values were initially based on Brodoefel and empirically optimized using three representative training sets. The inter- and intra-observer variability for the lumen and plaque volumes have been previously described.

Image Post-processing and Reconstruction

For prospective imaging, several phases will be transmitted to the MESA RC. In case of vessel wall calcification, additional images will be reconstructed using sharp-tissue convolution kernel and analyzed using a bone window setting to compensate for blooming artifacts. We anticipate the number of excluded segments to be <3%, and the number of image-quality excluded participants to

be <1%. Stented segments will be excluded from baseline and follow-up analysis for plaque and CAC measures.

DATA SAFETY AND MONITORING BOARD

The DSMB will meet via teleconference at six-month intervals during the course of the trial to monitor trial progress, safety and efficacy. The **report** includes extensive summaries by treatment group. Treatment groups will be coded for confidentiality (Group A and Group B) in the event there is significant adverse events ($p<0.05$) or significant benefit ($P<0.001$); the DSMB will be informed of the treatment codes prior to the end of the study. Along with a detailed commentary summarizing the results, the following statistical summaries by treatment arm will be provided:

- Treatment comparisons of all endpoints
- Summary of adverse events by type and level of severity
- Summary of study drug discontinuation
- Analyses of major endpoints by baseline subgroups

Schedule of Assessments

	Visit 1 (baseline)	Visit 2 (3 month)	Visit 3 (9 month)	Visit 4-phone only (15 month)	Visit 5 (18 month)
Informed Consent	X				
Demographics, Medical History	X				
Concomitant Medications	X	X	X		X
Blood pressure, height, weight	X	X	X		X
Blood draw	X		X		X
Physical Exam	X		X		X
CCTA	X		X		X
Dispense Study Drug	X	X	X		
Drug accountability		X	X		X
AE/ SAE assessment		X	X	X	X

Visit 1 (baseline):

The study staff will meet the prospective candidates at the study site. After study staff presenting the study and patient agreeing to participate, s/he will collect the following items:

1) Authorization for medical records release and informed consent,

2) Address, telephone number, social security number, phone numbers of contacts and physicians,

3) Demographic information including family history of CAD, smoking history, and medical history

4) Medication usage, including anti-platelets, prior or current use of cholesterol lowering medication, beta adrenergic blocking agents and other anti-hypertensives, calcium supplementation, hormone replacement and bisphosphonate use. All medications, including dietary supplements, and nutritional assessment will be measured to assess fish-oil intake.

5) Review of all inclusion/exclusion criteria.

6) If the patient meets all inclusion/exclusion criteria, will be scheduled for visit CCTA.

If the patient qualifies for the study and has signed informed consent, patient will undergo the following at this visit. If the patient is fasting and qualifies, the following can be performed on the same day, preferably in the morning and will be fasting for a minimum of 12 hours:

1) Blood pressure and pulse measurements from each arm

2) Height and weight measures

3) Laboratories – Phlebotomy (fasting) for markers of inflammation (Lp-PLA_2, hsCRP, IL-6), lipids and lipoproteins (ox-LDL, remnants [$VLDL_3$-C + IDL-C], apoliprotein A1 to remnant ratio, EPA, AA, EPA/AA ratio), genetic studies Additional blood storage (samples will be stored at -70 ^{0}C) for possible future testing (see Laboratory Analysis section below). Urinalysis and serum pregnancy testing (if appropriate) will be performed at this visit.

4) Physical examination

5) Coronary plaque volume/composition assessment using CT angiography

6) Re-Assessment of all medications, including dietary supplements, and nutritional assessment (dietary questionnaire), as well as allergies to contrast material and beta-blockers

7) Randomization to 1:1 to Vascepa or placebo.

The participants will then be randomly assigned to one of two treatment categories in a double-blind fashion. Randomization will occur according to a computer generated randomization code. Patients will receive a supply of randomized mediations, and be instructed to take medication each day. Group I will receive substance A and group II will receive substance B with same markings. Each bottle will have a 2-panel label

affixed, which will include the following information: study number, visit number, directions for use, emergency number. All analyses will be based upon intention to treat. The central laboratory assessment of fasting blood lipids will be determined throughout the study, and the central laboratory will be blinded as to patient treatment category.

After randomization, participants will return 3 months to assess compliance with medication, and receive an additional supply of medicine. Vital signs, blood samples, AE/ SAE/ MACE events will be also be assessed at the 3 month visit. At the 9 month visit, ALL baseline assessments will be repeated, inclusive of the cardiac CT angiography.

Interim evaluation will take place at 9 months and if efficacy is not achieved then participants will be followed for an additional 9 months, to determine lack of progression of low attenuation plaque volume. ALL baseline assessments will be repeated at 18 months, inclusive of the cardiac CT angiography.

At 15 months, a phone evaluation will take place, to assess AE/ SAE/ MACE events.

Laboratory Analyses

Upon entering the study, each subject will have blood drawn after a 12 hour fast. In all subjects, a blood sample of approximately 30 cc will be drawn from an arm vein by the phlebotomist. The sample(s) will be centrifuged and the serum and plasma separated. This will be placed into four tubes and aliquoted into 16 micro-vials for shipping and long-term storage. Locally, samples will be stored at -70 ^{0}C or below. Analyses will be performed to measure chemistry analysis, lipids and lipoproteins, as well as inflammatory markers. Frozen serum for coagulation factors, antioxidant levels, and hormonal levels

will potentially be evaluated for future studies/sub studies, and storage at -70^0C, will allow future studies. In addition to the above variables, biochemical and physiological parameters will be measured. At 9 months, fasting blood parameters, cardiac CT angiography and assessments will be made, and will be repeated again at the 18 month visit, as applicable.

Genetic Studies

If the subject consents to the optional genetic portion of this study, DNA analyses may be performed. These optional genetic analyses focus on inherited genetic variations such as Statin myopathy SLCO1b1, Clopidogrel Response(CYP2C19), ApoE-Isoform, Factor II, Factor V Leiden, MTHFR to evaluate their possible correlation to the disease and/or responsiveness to the therapies used in this study.

REFERENCES

1. Borow KM, Nelson JR, Mason RP. Biologic plausibility, cellular effects, and molecular mechanisms of eicosapentaenoic acid (EPA) inatherosclerosis. Atherosclerosis. 2015 Sep;242(1):357-66. doi: 10.1016/j.atherosclerosis.2015.07.035. Epub 2015 Jul 22.

2. von Schacky C, Angerer P, Kothny W, Theisen K, Mudra H. The effect of dietary omega-3 fatty acids on coronary atherosclerosis. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 1999;130:554–562.

3.Niki T,Wakatsuki T,Yamaguchi K,Taketani Y,Oeduka H,Kusunose K,Ise T,Iwase T,Yamada H,Soeki T,Sata M.Effects of the Addition of Eicosapentaenoic Acid to Strong Statin Therapy on Inflammatory Cytokines and Coronary Plque Components Assessed by Integrated Backcsatter Intravascular Ultrasound.Circulation Journal.2015.Advance Publication December 11, 2015.

4. Ando K,Watanabe T,Daidoji H,Otaki Y,Hashimoto N,Kumagai Y,Hashimoto N,Narumi T,Kadowaki S,Yamaura G,Wanezaki M,Funayama A,Yashiro Y,Iwayama T,Nishiyama S,Yakahashi H,Arimoto T,Shishido T,Miyamoto T,Kubota I.Combination Therapy of Eicosapentaenoic Acid and Pitavastatin for Coronary Plaque Regression Evaluated by Integrated Backscatter Intravascular Ultrasonography:A Randomized Controlled Trial.Circulation.2015:132:A12007.

5. Shintani Y,Kawasaki T.The Impact of a Pure-EPA Omega-3 Fatty Acid on Coronary Plaque Stabilization;A Plaque Component Analysis with 64-Slice Multi-Detector Row Computed Tomography.J Am Coll Cardiol.2012:59(13):E1713.

6. Koyama Y, Iwakura K, Ito H, et al. A case of borderline diabetic patient identified a quantitative regression and qualitative stability of isolated plaque by coronary CT angiography during purified eicosapentaenoic acid (EPA) administration [in Japanese]. *Ther Res*. 2010;31(2):219-225.

7. Burgstahler C, Reimann A, Beck T, Kuettner A, Baumann D, Heuschmid M, Brodoefel H, Claussen CD, Kopp AF, Schroeder S. Influence of a lipid-lowering therapy on calcified and noncalcified coronary plaques monitored by multislice detector computed tomography: Results of the new age ii pilot study. *Investigative radiology*. 2007;42:189-195.

8. Budoff MJ, Reuben Ram, Vahid Larinjani, Sachidanand Prabhu, Christopher Dailing, Nina Ram, Armand Ardestani, Naser Ahmadi, Reda Ibrahim, Jean Gregoire, Phillipe L L'Allier, Josephine Pressacco, Jean-Claude Tardif. Effect of Treatment with 5-Lipoxygenase Inhibitor VIA-2291 (Atreleuton) on Coronary Plaque Progression: A Serial CT Angiography Study. Circulation. 2012;126:A13826.

9. Matsumoto S, Nakanishi R, Alani A, Rezaeian P, Li D, Fahmy M, Abraham J, Dailing C, Flores F, Hamal S, Broersen A, Kitslaar P, Budoff MJ. The Effects Of Aged Garlic Extract On The Regression Of Coronary Plaque In Patients With Metabolic Syndrome: A Prospective Randomized Double-blind Study. J Nutr. 2016 Feb;146(2):427S-32S

10. Nakanishi R, Alani A, Budoff MJ. The Impact of Diabetes on Coronary Plaque Progression Assessed by a Novel Semi-Automated Quantitative Plaque Software on Coronary Computed Tomographic Angiography. J Am Coll Cardiol 2016; in press.

11. Zeb I, Li D, Nasir K, Malpeso J, Batool A, Flores F, Dailing C, Karlsberg RP, Budoff M. Effect of statin treatment on coronary plaque progression - A serial coronary CT angiography study. Atherosclerosis. 2013;231(2):198-204. doi: 10.1016/j.atherosclerosis.2013.08.019. PMID: 24267226

12. Gibson CM, Sandor T, Stone PH, Pasternak RC, Rosner B, Sacks FM. Quantitative angiographic and statistical methods to assess serial changes in coronary luminal diameter and implications for atherosclerosis regression trials. *American J Cardiol* 1992;69:1286-1290.

13. Hsieh FY, Bloch DA, Larsen MD. A simple method of sample size calculation for linear and logistic regression. *Statistics in medicine*. 1998;17:1623-1634

Appendix 1 –

Serious Adverse Events

A serious adverse event (SAE) is defined as an adverse event that meets **any** of the following criteria:

- Results in death
- Is life-threatening- Note: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
- Requires hospitalization or prolongation of existing hospitalization- Note: In general, hospitalization for treatment of a pre-existing condition(s) that did not worsen from baseline is not considered adverse events and should not be reported as SAEs.
- Results in disability/incapacity
- Is a congenital anomaly/birth defect;
- Is an important medical event- Note: Important medical events that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalizations, or the development of drug dependency.

Serious Adverse Event Reporting – Procedure for Investigators

1. Initial Reports

All SAEs occurring from the time of informed consent until 30 days following the last administration of study medication must be reported to the Sponsor or designee **within 24 hours** of the knowledge of the occurrence (this refers to any adverse event that meets any of the aforementioned serious criteria). SAEs that the investigator considers related to study medication occurring after the 30-day follow-up period will also be reported to the Sponsor or designee.

The investigator is required to submit SAE reports to the Institutional Review Board (IRB in accordance with local requirements. All investigators involved in studies using the same investigational medicinal product (IMP) will receive any Suspected Unexpected Serious Adverse Reaction (SUSAR) reports for onward submission to their local IRB as required. All reports sent to investigators will be blinded.

2. Follow-Up Reports

The investigator must continue to follow the patient until the SAE has subsided, or until the condition becomes chronic in nature, stabilizes (in the case of persistent impairment), or the patient dies. Within 24 hours of receipt of follow-up information, the investigator must update the SAE form electronically in the EDC system for the study and submit any supporting documentation (e.g., laboratory test reports, patient discharge summary, or autopsy reports) to the Sponsor or designee via fax or email.

3 Data Safety Monitoring Board (DSMB)

The adverse events will be monitored by an independent DSMB. A DSMB consisting of individuals with expertise in each of the efficacy areas (cardiac computed tomography, atherosclerosis) will be established. Dr Sion Roy will chair the DSMB, and will also be represented by Dr Muhlestein (cardiologist at IMH). DSMB will be independent from all staff involved in the day-to-day conduct of the study. The DSMB Chair will prepare interim reports to the DSMB on a regular basis, every 6 months or more often as it deems appropriate. Reports that include general study information such as accrual, dropout, and ineligibility rates and other performance parameters, will be available to the study leadership and will be discussed with them at open sessions. Data relating to safety and efficacy that are presented by treatment arm will be considered highly confidential and will be available only to the DSMB and the statistician preparing the reports, and will be discussed at closed sessions of the DSMB. The Interim reports to the DSMB will focus on analyses of the safety data -- primarily related to cardiac CT and study drug-- but other safety-related outcomes, such as CT related adverse effects, will also be reviewed in an unblinded fashion. Formal statistical comparisons on incidence of AEs, lab abnormalities, and concomitant medication use likely will lack power given the relatively small amount of patients. Will therefore use descriptive statistics for these data. The DSMB will decide on the basis of these data whether to recommend modifying or stopping the trial and will make recommendations to the sponsor and the study leadership.