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ATHEROTHROMBOSIS **I**NTERVENTION in **M**ETABOLIC SYNDROME with Low **H**DL/HIGH TRIGLYCERIDE and **I**MPACT ON **G**LOBAL **H**EALTH OUTCOMES

AIM-HIGH

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LIST OF ABBREVIATIONS

ABI	**Ankle-Brachial Index**
ACC	**American College of Cardiology**
AIM-HIGH	**Atherothrombosis Intervention in Metabolic Syndrome with low HDL/high TG and Impact on Global Health Outcomes**
ATP III	**Adult Treatment Panel III**
CABG	**Coronary Artery Bypass Graft**
CAD	**Coronary Artery Disease**
CHD	**Coronary Heart Disease**
CEC	**Clinical Events Committee**
CHF	**Congestive Heart Failure**
CK	**Creatine Kinase**
CRP	**C-Reactive Protein**
CT	**Computed Tomography**
CTC	**Clinical Trial Coordinating Center**
DM	**Diabetes Mellitus**
DSMB	**Data and Safety Monitoring Board**
ECG	**Electrocardiogram**
ERN	**Extended-Release Niacin**
FATS	**Familial Atherosclerosis Treatment Study**
HATS	**HDL Atherosclerosis Treatment Study**
HDL	**High-Density Lipoprotein**
LAD	**Left Anterior Descending**
LDL	**Low-Density Lipoprotein**
Lp(a)	**Lipoprotein (a)**
MI	**Myocardial Infarction**
MRI	**Magnetic Resonance Imaging**
NCEP	**National Cholesterol Education Program**
NSTE	**Non-ST-Segment Elevation**
PAD	**Peripheral Arterial Disease**
PCI	**Percutaneous Coronary Intervention**
PTCA	**Percutaneous Transluminal Coronary Angioplasty**
TG	**Triglyceride**
ULN	**Upper Limit of Normal**
VLDL	**Very-Low Density Lipoprotein**

1. EXECUTIVE SUMMARY (See Follow-Up After Blinded Treatment Phase, Amendment 6, below on page 9)

Title	**AIM-HIGH Trial: *A*therothrombosis *I*ntervention in *M*etabolic Syndrome with Low *H*DL/High Triglycerides and *I*mpact on *G*lobal *H*ealth Outcomes**
Objectives	Primary: In patients with established vascular disease and atherogenic dyslipidemia, we plan to compare the efficacy and safety of statin monotherapy (with simvastatin) versus combination therapy (with extended-release niacin plus simvastatin), at comparable levels (<80 mg/dL [2.1 mmol/L]) of on-treatment LDL-C, in reducing the risk for the composite endpoint of coronary heart disease (CHD) death, nonfatal myocardial infarction (MI), ischemic stroke, hospitalization for acute coronary syndrome (ACS), or symptom-driven coronary or cerebral revascularization. Secondary: • To evaluate the effect of therapy on the composite endpoint of CHD death, non-fatal MI, high-risk ACS, or ischemic stroke • To evaluate the effect of therapy on the composite endpoint of CHD death, non-fatal MI, or ischemic stroke • To evaluate the effect of therapy on cardiovascular mortality Tertiary: • To evaluate the effect of therapy on total mortality • To evaluate the effect of therapy on the composite endpoint of, and the individual components and subcomponents of the composite endpoint of, death, non-fatal MI, stroke, hospitalization for acute coronary syndrome, or any arterial revascularization • To evaluate the effect of therapy for preventing clinical events, as defined above, among patients meeting current criteria for metabolic syndrome as defined by the NCEP ATP III, or future criteria for metabolic syndrome as they may evolve, or diabetes • To assess the effects of statin monotherapy versus combination therapy on lipids and lipoproteins, including, apoA-I, apoB, apoC-III, Lp(a), HDL subfractions/particle

	size, LDL size and subclass distribution, and their independent contribution to predicting outcomes • To assess the effect of therapy on inflammatory markers such as C-reactive protein and fibrinogen, and their independent contribution to predicting outcomes
Design	This is a multicenter, prospective, randomized, double-blind, parallel-group, active comparator study.
Study Population	**INCLUSION CRITERIA (see additional detail in protocol):** Men and women aged 45 and older with the following two criteria: ***1. Established Vascular Disease, Defined as One or More of the Following:*** **a. Documented coronary artery disease** (CAD; one or more of the following primary criteria must be satisfied): • Documented multivessel CAD (one or more ≥ 50% stenoses in *two* major epicardial coronary arteries – with or without antecedent revascularization) • Documented history of MI • Hospitalization for unstable angina with objective evidence of ischemia (ST-segment deviation or biomarker positivity) **b. Documented cerebrovascular or carotid disease** (one of the following primary criteria must be satisfied): • Documented previous ischemic stroke • Symptomatic carotid artery disease with ≥ 50% carotid arterial stenosis • Asymptomatic carotid artery disease with ≥ 70% carotid arterial stenosis • History of carotid revascularization (catheter-based or surgical) **c. Documented Peripheral Arterial Disease** (PAD; one or more of the following primary criteria must be satisfied): • ABI < 0.85 with or without symptoms of intermittent claudication • History of aorto-iliac or peripheral arterial intervention (catheter-based or surgical)

	*2) **Dyslipidemia defined as*** (all 3 must be satisfied): • The equivalent, off lipid therapy, of: - LDL-C of ≤ 180 mg/dL (4.7 mmol/L) - HDL-C of ≤ 40 mg/dL (1.0 mmol/L) [men] or ≤ 50 mg/dL (1.3 mmol/L) [women] - TG ≥ 150 mg/dL (1.7 mmol/L) and ≤ 400 mg/dL (4.5 mmol/L) • For patients entering the trial on a statin ± ezetimibe: - the upper limit for LDL-C is adjusted according to the specific statin (± ezetimibe) and statin-dose (see Table, section 4.4.1.2) - HDL-C of ≤ 42 mg/dL (1.1 mmol/L) [men] or ≤ 53 mg/dL (1.4 mmol/L) [women] - TG ≥ 100 mg/dL (1.1 mmol/L) and ≤ 400 mg/dL (4.5 mmol/L) **Major Exclusion Criteria** (see additional detail in protocol): • Coronary Artery Bypass Graft (CABG) surgery within 1 year of planned enrollment (run-in phase) • Percutaneous Coronary Intervention (PCI) within 4 weeks of planned enrollment (run-in phase) • Hospitalization for acute coronary syndrome and discharge within 4 weeks of planned enrollment (run-in phase) • Fasting glucose >180 mg/dL (10 mmol/L) or hemoglobin A1C >9% • For patients with diabetes, inability or refusal to use a glucometer for home monitoring of blood glucose
	Rescreening • Patients disqualified for enrollment in the study by virtue of the above inclusion/exclusion criteria may subsequently be rescreened and considered for enrollment if at a later time they no longer fail to meet those criteria of if disqualifying exclusion criteria are corrected
Total expected number of clinical centers and subjects	• A minimum of 54 clinical centers in the United States and Canada will be involved in this study • Estimated sample size of 3,300 subjects

Participating physicians/sites	The recruitment of the sites and subjects should allow an extrapolation of the results to the broadest possible population with vascular disease and atherogenic dyslipidemia. Therefore the recruitment of the sites and subjects will be done carefully in order to ensure representation of the overall population. To achieve this goal two rules will be followed: • Pre-defined selection of physicians and sites • Prospective and consecutive enrollment of subjects
Main data collected	• Baseline demographic information, employment status, medical history, physical examination, current medical treatments • Post-randomization, clinical events including all causes of death, MI, stroke, vascular interventions and hospitalizations, with dates and detailed documentation, since last visit • Corresponding secondary and tertiary efficacy endpoint parameters, as appropriate • Fasting blood lipids and lipoproteins • Safety endpoints, including fasting blood glucose, creatinine hemoglobin A1C, thyroid function test, liver function tests
Statistical analysis	• Randomization will be stratified by site, gender and prior history of diabetes • Intent-to-treat analysis. • Parameters will be summarized using mean, median, standard deviation for continuous data and percentage for categorical data • Survival analysis using Cox Proportional Hazards analysis of primary efficacy outcome comparing the 2 groups (combination versus monotherapy) • Statistical analyses for the efficacy outcomes will be performed at the 2.5% significance level using 1-sided tests. All other statistical analyses will be performed at the 5% significance level using 2-sided tests. • Interim analyses are planned using group sequential methods to monitor the trial The analysis is planned to: • Describe at baseline subject characteristics, including lipids and lipoproteins, stroke history, cardiovascular risk factors, diabetes mellitus, or metabolic syndrome, among others • Compare the primary, secondary, and tertiary endpoints

	between the patient groups receiving the combination anti-dyslipidemic therapy and statin monotherapy at corresponding follow-up time points
Timelines	3,300 qualified patients will be enrolled in a minimum of 54 clinical sites over a planned 2 year period with a mean follow-up of 4 years
Follow-up After Blinded Treatment Phase (Amendment 6, June 1, 2011)	On May 4, 2011, as a consequence of a carefully considered recommendation from the independent AIM-HIGH DSMB, NHLBIdecided to stop double-blind therapy with extended-release niacin or placebo. The NHBLI made the decision because the data showed that there was less than a 1 in 10,000 chance that the trial would ever show a significant benefit on the primary composite outcome measure defined above. Per DSMB recommendation, participants will be followed for an additional 18 months for the endpoint events described above. There will be no study drug provided; lipid management will be the responsibility of the participant's personal physician.

2. Background and Rationale for AIM-HIGH (See 2.3.1, DSMB Recommendation to Discontinue Double-Blind Therapy below on page 15)

2.1 Vascular disease, atherogenic dyslipidemia and metabolic syndrome, statins as monotherapy and combination therapy in CHD management

2.1.1 Vascular disease

Coronary heart disease remains the leading cause of death and disability in the U.S. and the Western world. Data from the *2002 Heart and Stroke Statistical Update*, American Heart Association, indicate that there are 12.6 million individuals in the U.S. with a history of MI, angina, or both. The prevalence of stroke, transient ischemic attack (TIA) and PAD in the U.S. currently is 4.6 million, 4.9 million and 8-12 million, respectively. Aggregate direct and indirect costs for CHD in the U.S. in 2001 were $112 billion and for stroke/TIA were $49 billion.[1]

The pathologic basis of symptomatic vascular disease including CHD, cerebral vascular disease and PAD is atherothrombosis, which is characterized by an unpredictable, sudden rupture/fissure of an atherosclerotic plaque. A rupture or large fissure of an atherosclerotic plaque typically results in a large thrombus formation, which in turn results in an acute ischemic event such as myocardial infarction or ischemic stroke. A small fissure may result in a mural thrombus, which may cause transient ischemia such as unstable angina or TIA.

2.1.2. Atherogenic Dyslipidemia and Metabolic Syndrome

Dyslipidemia is one of the major modifiable risk factor of atherosclerosis.[2] Elevated plasma concentrations of the apolipoprotein B (apo B)–containing low-density lipoproteins (LDL), very low-density lipoproteins (VLDL) and their remants, and lipoprotein (a) [Lp(a)] promote the development of atherosclerotic lesions, while elevated levels of HDL-C inhibit plaque formation.[3] The type of LDL particles present in the blood may also be a key atherogenic risk factor, with small, dense LDL particles more likely to be involved in the formation of plaques than larger, more buoyant ones.[4,5]

An increasingly common dyslipidemia seen among patients with established vascular disease consists of a low HDL-C together with elevated triglycerides and preponderance of small, dense LDL particles, so-called 'atherogenic dyslipidemia.' Low-density lipoprotein cholesterol may be only minimally elevated. This phenotypic pattern is characteristic of patients with diabetes or metabolic syndrome, the presence of each of which significantly increases the risk for CHD. In fact, the great majority of patients with established vascular disease and atherogenic dyslipidemia will have at least one other component of the metabolic syndrome (BG Brown, personal communication).

Metabolic syndrome was identified in 1988 and defined as a combination of insulin resistance, hyperinsulinemia, increased plasma levels of triglycerides, and decreased

plasma levels of HDL-C.[5,6] Insulin resistance in the metabolic syndrome occurs at the level of glucose and free fatty acid metabolism, and the lipoprotein abnormalities consist of increases in plasma levels of triglycerides, apo B, and smaller denser LDL particles, with marked reductions in plasma levels of HDL-C and apo A-I.[6,7]

Based on the Third National Health and Nutrition Examination Survey (NHANES),[7] the estimated overall prevalence of the metabolic syndrome in the United States is 24% (43% of men and women ≥50 years old), which corresponds to approximately 47 million individuals. Coupled with the metabolic syndrome is a potentially increased risk for the development of diabetes and coronary artery disease.[9-13]

2.1.3. Statin monotherapy in vascular disease management

The etiologic role of elevated blood levels of LDL-C in atherosclerosis has long been established by both its strong association with CHD in well-characterized populations and the unequivocal therapeutic benefit of drug therapies that specifically reduce its concentrations. In primary and secondary prevention trials using a statin, plasma LDL-C was reduced by 25%-36% and coronary event rates were reduced by 24%-34% compared with placebo. Most recently, the Heart Protection Study (HPS) randomized over 20,000 patients with CHD, occlusive arterial disease, or diabetes to treatment with simvastatin or placebo for an average of 5 years.[14] Simvastatin treatment was associated with a mean 29% decrease in LDL-C compared to placebo and relative risk reductions in non-fatal myocardial infarction (MI) or CHD death of 27% (p<0.0001), non-fatal or fatal stroke of 25% (p<0001), and coronary or non-coronary revascularization of 24% (P<0001). Furthermore, these benefits were independent of gender, age, baseline risk status.

It is important to note that *although these trials demonstrated an approximate 30% relative reduction in cardiovascular risk, patients treated with a statin, even those who achieved on-trial LDL-C levels $\leq$ 70 mg/dL (1.8 mmol/L), still experienced an event rate equal to at least 60% of the rate seen in those treated with placebo*.[15] For example, in the recently-reported Pravastatin or Atorvastatin Evaluation and Infection Trial (PROVE-IT) comparing atorvastatin 80 mg versus pravastatin 40 mg in patients hospitalized for an acute coronary syndrome, the 2-year risk for a major cardiovascular event (death from any cause, MI, documented unstable angina requiring re-hospitalization, or revascularization) among atorvastatin-treated patients, who achieved a mean on-trial LDL-C of 62 mg/dL (1.6 mmol/L), was 22%. For the endpoint of death or MI, the 2-year event rate in the atorvastatin group was 8%. Extrapolated over a 10-year period, the risk for death or MI would be as high as 40% in this group, despite having a mean LDL-C of 62 mg/dL (1.6 mmol/L). These figures point to the fact that, among patients treated with statins as monotherapy for dyslipidemia, the residual risk for an event is unacceptably high. Clearly, LDL-C reduction alone is insufficient to optimize CHD management.

2.1.4. Combination Therapy in CHD management

2.1.4.1 Importance of low HDL-C in dyslipidemia

Risk assessment limited to LDL-C fails to identify a substantial number of patients at risk for coronary events and other vascular events. Patients with metabolic syndrome and most patients with type 2 diabetes have multiple lipid abnormalities: increased plasma triglycerides and apoB levels, increased number of smaller denser LDL particles and decreased plasma HDL-C levels. Most patients with CHD also have multiple lipid abnormalities. The Veterans Affairs HDL Intervention Trial (VA-HIT) group[16] found that 87% of 8500 patients with established CHD had suboptimal LDL-C levels (≥100mg/dL [2.6 mmol/L]), 33% had hypertriglyceridemia (triglycerides levels > 200mg/dL [2.3 mmol/L]), and approximately 60% had low levels of HDL-C (≤40mg/dL [1.0 mmol/L]).

Low levels of HDL-C are strong independent predictors of CHD risk. Each 1mg/dL [0.03 mmol/L] increase in HDL-C is associated with a 2% to 3% decrease in CHD risk, even after adjustment for other risk factors, and predicts coronary risk regardless of LDL-C levels.[17] The NCEP III has identified HDL-C levels less than 40mg/dL (1.0 mmol/L) as a risk factor for CHD, although no goal has been set.[2] Subsequently, multivariate analyses of clinical trials in hypercholesterolemic patients have shown that raising HDL-C levels was associated with reductions in CHD events. VA-HIT was the first study to provide conclusive evidence that raising low levels of HDL-C, in CHD patients with normal LDL-C levels, was associated with significant reductions in coronary events.[18] More recently, the HDL Atherosclerosis Treatment Study (HATS) has shown that treatment of the total lipid profile with a combination of simvastatin and niacin was associated with a significant regression of coronary atherosclerosis and further reductions in clinical events[19].

2.1.4.2 Emerging role of combination therapy

The five classes of lipid-modifying agents (statins, fibrates, bile acid sequestrants, ezetimibe and niacin) produce their major effect on one lipid or lipoprotein but have only moderate or minor effects on the others.[1] Therefore, each drug as monotherapy may leave a large number of patients treated inadequately. In contrast, combination therapy can provide more effective coverage of the entire lipid profile. The clinical importance of combination therapy is underscored by the high prevalence of low HDL-C in patients with CHD, metabolic syndrome and diabetes. Review of previous trials that combined various statins with preparations of sustained- or immediate-release niacin have not only shown the beneficial effects on improving dyslipidemia[19,20] but also on facilitating the regression of the atherosclerotic lesions and reducing clinical events including death, MI or revascularization.[21,22] Results of recent trials of combination therapy with statins and niacin have demonstrated improved regulation of dyslipidemia.[23,24]

2.2 Niacin and anti-dyslipidemic therapy

2.2.1 Mechanism of action

Niacin has favorable effects on all major lipids and lipoproteins. Its mechanisms of action are not completely understood. Niacin has a significant effect on HDL-C levels. The primary mechanism by which niacin increases HDL-C is by reducing the catabolic rate of apolipoprotein (apo) AI, the major protein carrier of HDL.[25,26]. Reverse cholesterol transport is thereby enhanced as cholesterol-deficient, apolipoprotein A-I-containing HDL particles are re-circulated to peripheral cells to transport additional cholesterol to the liver.

Niacin also produces large and rapid reductions in TG and inhibits its hepatic esterification, thereby reducing production of atherogenic lipoproteins[35]. It inhibits hormone-sensitive lipoprotein lipase in fat cells reducing intracellular lipolysis and release of fatty acids into the plasma. The decrease in circulating free fatty acids reduces uptake by the liver, thereby inhibiting hepatic VLDL production. Since VLDL is converted into intermediate-density lipoprotein and then LDL, reductions in VLDL lower LDL-C.[26]

2.2.2 Niacin in combination therapy

Several studies have evaluated the role of the combination therapy of niacin and statin. In one study using fluvastatin, combination therapy with immediate-release (IR) niacin produced greater reductions in LDL-C than did combination therapy with placebo (40% vs 25%, $p<0.001$).[27] In another study, the combination of IR niacin and simvastatin had no greater effect on LDL-C than simvastatin alone; however, HDL cholesterol increased by 31%, compared with 13% for the statin alone group ($p<0.05$).[28] In a third study, combination therapy of 1g/day once-daily niacin extended-release (Niaspan®) and statin lowered LDL-C by an additional 8% and increased HDL-C by 24%; combination therapy of 2g/day extended-release niacin and statin lowered LDL-C an additional 20% and raised HDL-C an additional 27%.[29] In a recent study evaluating the efficacy of the combination of the extended-release niacin and rosuvastatin, compared with rosuvastatin alone, rosuvastatin 10mg/ER niacin 2 g produced significantly greater increases in HDL cholesterol (11% vs 24%, $p<0.001$) and apolipoprotein A-I (5% vs 11%, $p<0.017$).[30]

2.2.3 Safety and tolerability of niacin use

General population

Despite the lipid and cardiovascular benefits associated with niacin, its use has been limited in clinical practice by poor tolerability caused by dose-dependent side effects, particularly cutaneous and gastrointestinal complaints associated with immediate-release or crystalline niacin.[31] Almost all patients who take immediate-release niacin experience flushing, which leads to medication discontinuation in approximately 10% to 20% of subjects in clinical studies.[32,33]. Elevated hepatic transaminase levels have been reported

with immediate-release niacin, usually after long-term use with high doses (>3 to 4 g/d),[34-36], but hepatic failure has been rare.[35,37]

Sustained-release preparations of niacin were developed to overcome the limitations associated with the immediate-release form.[32] The different toxicologic characteristics of immediate-release and sustained-release preparations are due to the dual pathways of niacin metabolism—a low-affinity, high-capacity conjugative pathway that leads to flushing and a high-affinity, low-capacity nonconjugative pathway that may lead to hepatotoxicity. Recently, a once-daily extended-release niacin (Niaspan®, Kos Pharmaceuticals, Miami, FL) has been formulated to distribute drug absorption over an intermediate time of 8 to 12 hours[38] to balance metabolism between both pathways. In one study, extended-release niacin once-daily was shown to have efficacy equivalent to immediate-release niacin three times daily and to reduce episodes of flushing by about 80%.[39] In a 96-week study, doses of 2000 mg/d of extended-release niacin, reduced LDL-C, triglycerides, and Lp(a) by 18%, 24%, and 36%, respectively, while increasing HDL-C by 29% from baseline.[40] Reversible elevations in liver function tests greater than 3 times upper limit of normal (ULN) occurred in <1.0% of patients, and serious hepatic toxicity was not evident.

In patients with diabetes mellitus (DM)

Niacin appears ideally suited to treating the atherogenic dyslipidemia associated with diabetes, but traditionally niacin use was thought to be relatively contraindicated in patients with diabetes due to adverse effects on glucose control and insulin sensitivity.[41,42]. However, due to the high prevalence of low HDL-C in diabetes and the difficulty of raising low HDL-C levels with other agents, several recent studies have re-evaluated the use of niacin in patients with controlled type 2 diabetes. The Arterial Disease Multiple Intervention Trial (ADMIT) evaluated the effect of niacin in 468 patients with peripheral arterial disease, including 125 patients with diabetes[43]. Niacin produced small increases from baseline in average glucose levels among patients with diabetes (8.1 mg/dL [0.45 mmol/L]; p=0.04) and without diabetes (6.3 mg/dL [0.35 mmol/L]; p<0.001), but hemoglobin A1C levels were not significantly changed from baseline. These small glycemic changes did not increase niacin discontinuation or alter hypoglycemic therapy compared with placebo. Similar results were seen in the Assessment of Diabetes Control and Evaluation of the Efficacy of Niaspan Trial (ADVENT) which randomized 148 diabetic patients to treatment with an extended-release niacin (niacin ER) 1000 mg or 1500 mg or placebo.[44]

Moreover, in a post-hoc analysis from the HDL Atherosclerosis Treatment Study (HATS), niacin/simvastatin combination therapy produced nearly a 50% relative reduction in major clinical events in the subset of patients with diabetes or impaired fasting glucose.[45] There was no significant difference in glycemic control between the active treatment or placebo groups. These results suggest that niacin can be used safely in diabetic patients. Although glucose levels should be monitored for potential hyperglycemia and additional glycemic control may be needed, this risk is offset by the potential cardiovascular benefits resulting from the broad improvement in the lipid triad.

Thus, niacin may be considered as an alternative to statins and fibrates when patients with diabetes cannot tolerate these agents or when their hypertriglyceridemia or low HDL-C levels do not sufficiently improve.[43]

2.3 AIM-HIGH Rationale

The hypothesis of AIM-HIGH is that combination anti-dyslipidemic therapy (extended-release niacin plus simvastatin) will be superior to statin monotherapy alone (simvastatin) when used as secondary prevention in reducing long-term clinical events in patients with documented vascular disease and atherogenic dyslipidemia. Based on these selection criteria, the vast majority of these patients are anticipated to satisfy current NCEP ATP III criteria for a diagnosis of metabolic syndrome.

To date, there have been several large randomized controlled trials involving statin monotherapy versus placebo to reduce elevated LDL-C and clinical events in CHD patients, but only one secondary prevention randomized controlled trial to assess the role of raising low levels of HDL-C and/or lowering TG levels and its impact on favorably reducing CHD death, MI and stroke (VA-HIT). VA-HIT clearly demonstrated the superiority of gemfibrozil versus placebo in male veterans with CHD and low levels of HDL-C, but was limited in its overall generalizability because women were excluded. In addition, the increase in HDL-C in that study was quite modest (~6%) compared to what is anticipated with niacin.

Thus, while VA-HIT provides important "proof of concept" that the "HDL hypothesis" of therapeutically raising low levels of HDL-C reduces coronary and cerebrovascular events during long-term follow-up, there has been, to date, no randomized controlled trial that has evaluated prospectively the role of "combination dyslipidemic therapy" in a more geographically and demographically-diverse population of men and women with vascular disease manifested as CHD, CVD or PAD and who have the increasingly common lipid profile of low HDL-C, elevated triglycerides (with or without elevated LDL-C), and features of the insulin resistance (metabolic) syndrome. The current gaps in our scientific knowledge and contemporary therapeutics as to how such patients should be managed optimally are large, and the proposed AIM-HIGH trial seeks to address these important considerations.

2.3.1 DSMB Recommendation to Discontinue Double-Blind Therapy (Amendment 6, Revision June 1, 2011)

On May 4, 2011, based on a carefully considered recommendation from the independent AIM-HIGH DSMB, the decision was made to stop double-blind therapy with extended-release niacin or placebo. The decision to discontinue double-blind therapy was reached because the data showed that there was less than a 1 in 10,000 chance that the trial would ever show a significant benefit on the primary outcome measure, that is, CHD death, non-fatal myocardial infarction, ischemic stroke, hospitalization for acute coronary syndrome

or symptom driven coronary or cerebral revascularization. The pre-established boundary for demonstration of lack of efficacy was crossed in the analysis of the data for a scheduled interim look by the DSMB, as provided for in the Interim Analysis Plan.

The DSMB also observed that while the total number of strokes was low, the data showed small imbalance in the occurrence of ischemic strokes which reached a nominal level of statistical significance. In AIM-HIGH, 40 participants had an ischemic stroke; 28 of those occurred in the extended-release niacin group (although 9 of these participants had discontinued treatment with extended-release niacin at least two months and up to four years prior to the event). Further analyses are being conducted to explore this unexpected increase in ischemic stroke. No similar finding has been observed in previous studies with any form of niacin therapy for any length of follow-up. Moreover, previous studies have consistently shown reduction in stroke associated with niacin administration.

Protocol amendment 6 provides for continuing follow-up of patients for 18 months after stopping double-blind therapy with extended-release niacin or placebo to ascertain clinical events, as recommended by the DSMB. The participant's personal physician will be responsibility for lipid management.

3. Study Objectives (See 3.4 Objective for Continuing Patient Follow-up for 18 Months after Stopping Double-Blind Therapy, below on page 17)

3.1 Primary Objective:

To assess, during a 3-5 year follow-up, the comparative efficacy and safety of statin monotherapy (simvastatin) versus combination therapy (niacin extended-release plus simvastatin), at comparable levels ($\leq$80 mg/dL [2.1 mmol/L]) of on-treatment LDL-C, in reducing the risk for clinical events (CHD death, nonfatal MI, ischemic stroke, hospitalization for acute coronary syndrome, symptom-driven coronary or cerebral revascularization) in vascular disease patients with atherogenic dyslipidemia (low HDL-C and high triglycerides).

3.2 Secondary Objectives

- To evaluate the effect of therapy on the composite endpoint of CHD death, non-fatal MI, hospitalization for high-risk ACS, or ischemic stroke

- To evaluate the effect of therapy on the composite endpoint of CHD death, nonfatal MI, or ischemic stroke

- To evaluate the effect of therapy on cardiovascular mortality

3.3 Tertiary Objectives

- To evaluate the effect of therapy on total mortality

- To evaluate the effect of therapy on the composite endpoint of, and the individual components and subcomponents of the composite endpoint of death, non-fatal MI, stroke, hospitalization for acute coronary syndrome, or any arterial revascularization

- To evaluate the effect of therapy for preventing clinical events, as defined above, among patients meeting current criteria for metabolic syndrome as defined by the NCEP ATP III, or future criteria for metabolic syndrome as they may evolve, or diabetes

- To assess the effects of statin monotherapy versus combination therapy on lipids and lipoproteins, including apoA-I, apoB, apoC-III, Lp(a), HDL subfractions/particle size, LDL size and subclass distribution, and their relationship to outcome

- To assess the effects of therapy on inflammatory markers, such as C-reactive protein and fibrinogen, and their relationship to outcome

3.4 Objective of Extending Participant Follow-up for 18 Months after Stopping Double-Blind Therapy (Amendment 6, Revision June 1, 2011)

The objective of this continuing follow-up is to calculate the incidence of primary endpoint events and components of the primary endpoint, including stroke after discontinuation of double-blind lipid therapy.

4. Study Design

4.1 General review (See 4.1.1 Discontinuation of Double-Blind Therapy with Extended-Release Niacin or placebo below on page 18)

- Multicenter, prospective, randomized, double-blind, parallel-group, active comparator design of statin monotherapy (simvastatin) versus combination anti-dyslipidemic therapy (extended-release niacin plus simvastatin) in high-risk patients with established vascular disease (i.e., those who have a 10-year risk of an event of ≥20%) who have atherogenic dyslipidemia (low HDL-C and high triglycerides). The vast majority of these patients will qualify for a diagnosis of metabolic syndrome.

- Prospectively, eligible patients with documented vascular disease will undergo screening to establish suitability for inclusion in the trial. For patients currently treated with a statin ± ezetimibe, no drug washout will be performed. All other lipid-altering drugs (e.g., niacin, fibrates, resins) must be discontinued at least 4 weeks prior to the qualifying lipid determination. Lipid inclusion criteria are:

untreated or off-therapy LDL-C ≤180 mg/dL [4.7 mmol/L]; HDL-C ≤40 mg/dL (1.0 mmol/L) [men] or 50 mg/dL (1.3 mmol/L) [women]; and TG ≥ 150 mg/dL (1.7 mmol/L) and ≤400 mg/dL (4.5 mmol/L). For statin-treated patients (+ ezetimibe), the upper limit for LDL-C is adjusted according to the specific statin and dose (section 4.4.1.2). In addition, the HDL-C and TG entry criteria for those on statins are modified to: HDL-C of ≤ 42 mg/dL (1.1 mmol/L) [men] or ≤ 53 mg/dL (1.4 mmol/L) [women] – assumes an average statin effect of about +5%; TG ≥ 100 mg/dL (1.1 mmol/L) and ≤ 400 mg/dL (4.5 mmol/L) – assuming a statin effect of up to –33%. Since ezetimibe has minimal effects on TG and HDL-C, threshold criteria for these variables are not different from those in patients taking statin alone.

4.1.1 Follow-up After Discontinuation of Double-Blind Therapy with Extended-Release Niacin or Placebo (Amendment 6, Revision June 1, 2011)

One the basis of the recommendation of the DSMB made on May 4, 2011, double-blind therapy with extended-release niacin or placebo will be discontinued as of May 25, 2011. A final follow-up visit for the double-blind treatment phase of the study will be scheduled for each patient between June 1, 2011 and August 15th, 2011. Patients will then be followed for an additional 18 months with lipid management at the direction of their personal physician. No study drug or intervention will be provided during this extended follow-up period. Follow-up will consist of one telephone call at 9 months and one final in-clinic visit at 18 months after the visit that occurs between June 1, 2011 and August 15th, 2011 and marks the end of the double-blind therapy portion of the trial.

4.2 Study committees

Executive Committee

The Executive Committee of the study is composed of a core group of investigators/academic members from participating clinical centers. A representative of the industry sponsor and the principal investigator of the central laboratory will be ex-officio members. This committee will provide scientific and strategic direction for the trial and will have overall responsibility for the design, execution, and publication. Detailed responsibilities and membership for this committee will be provided as needed. The Executive Committee of AIM-HIGH will be in charge of the logistical coordination of the different study committees.

Clinical Event Committee (CEC)

The CEC is composed of multidisciplinary academic members. This committee will be responsible for blindly validating all the primary and secondary efficacy outcome events reported by the investigators. This committee will create a charter with details on the methods and assessment of clinical events and their precise definitions.

Data and Safety Monitoring Board (DSMB)

A Data Safety Monitoring Board (DSMB) will be instituted for this study in order to ensure its ongoing safety and to oversee the Interim Analyses. Recommendation for trial continuation will be guided by monitoring boundaries at interim analyses at which formal efficacy analysis is performed as well as safety evaluations at all safety data reviews.

Members of the DSMB will not be otherwise participating in the trial. The committee will include at least one cardiologist with expertise in atherosclerosis and inflammatory processes, one lipidologist and diabetologist as well as an independent statistician. A DSMB Charter will be drafted and approved by the DSMB, the NHLBI and the Executive Committee. The Charter will provide details regarding the interim analysis and monitoring plan. Safety review meetings will be held approximately every 6 months. Safety data will include pre-specified evaluation of parameters for blood glucose, myopathy, hepatotoxicity as well as other possible clinical side effects such as gout, as requested by the DSMB. Formal interim analyses for efficacy data will be performed as per separate DSMB charter. Enrollment to the study will continue throughout the scheduled meetings of the DSMB.

4.3 Randomization and duration of study participation

About 3,300 patients (1,650 in each group) will be randomized to receive simvastatin monotherapy or niacin extended-release plus simvastatin. As described in Section 5.2, the estimated study duration that served as the hypothesis for sample size calculations comprises a planned 2-year enrollment and a mean 4 years of follow-up. In any case, all randomized patients will be followed until study end date, with a minimum follow-up duration of three years and a maximal follow-up duration that corresponds to the time between the first randomization and the study end date.

4.4 Selection of patients

4.4.1 Inclusion criteria:

Men and women aged 45 and older with established vascular disease and atherogenic dyslipidemia, defined in the following ways:

4.4.1.1 Established Vascular Disease

a. Documented CAD (one or more of the following primary criteria must be satisfied):

- Documented multivessel CAD, defined as one or more $\geq$ 50% stenoses in at least *two* major epicardial coronary arteries by angiography. Patients in whom percutaneous coronary intervention (PCI) has been successfully performed on one or both coronary stenoses - even if there is no residual post-PCI stenosis - will still be considered to satisfy the trial eligibility criterion of multivessel CAD

- Documented previous MI (two of the following three criteria must be satisfied):
  - Characteristic ischemic chest pain or pain in associated referral areas
  - Elevation of CK (at least twice the upper limit of normal values) and/or CK-MB (at least twice the upper limit of normal values) and/or troponin T or I (at least twice the upper limit of normal value)
  - Development of Q waves in at least two adjacent ECG leads, or development of a new dominant R wave in V1

- Hospitalization for NSTE acute coronary syndrome with objective evidence of ischemia (ST-segment deviation or biomarker positivity) – stable for at least 4 weeks following hospital discharge. Patients must have clinical findings of ischemic symptoms consistent with angina (chest or mid-epigastric discomfort, dyspnea, or symptoms that represent an "anginal equivalent," if atypical) in the judgment of the investigator

b. Documented cerebrovascular or carotid disease (one of the following primary criteria must be satisfied):

- Documented previous ischemic stroke (all criteria must be satisfied):
  - A focal ischemic neurological deficit persisting for more than 24 hours
  - Considered to be of ischemic origin
  - Onset within previous 5 years but not within 8 weeks prior to enrollment
  - Patients with history of ischemic stroke and atrial fibrillation do *not* satisfy the criterion for CVD, in the absence of other evidence for cerebrovascular disease. Patients with history of ischemic stroke and sinus rhythm *are* eligible

A CT scan or MRI must have been performed to rule out hemorrhage and non-ischemic neurological disease.

- Symptomatic carotid artery disease with ≥50% stenosis established by angiography or color-coded duplex ultrasound on the basis of recognized criteria (see Appendix 2 for method of evaluation)

- Asymptomatic carotid stenosis ≥70% established by angiography or color-coded duplex ultrasound on the basis of recognized criteria (see Appendix 2 for method of evaluation)

- History of carotid revascularization (surgical or catheter-based)

c. Documented PAD (one or more of the following primary criteria must be satisfied):

- ABI <0.85, with or without symptoms of intermittent claudication (see Appendix 3 for measurement method)

- A history of aorto-iliac or peripheral arterial intervention (catheter-based or surgical)

And

4.4.1.2 Atherogenic Dyslipidemia defined as:

- Off therapy, the following criteria must all be met:

  - LDL-C of ≤ 180 mg/dL (4.7 mmol/L)
  - HDL-C of ≤ 40 mg/dL (1.0 mmol/L) [men] or ≤ 50 mg/dL (1.3 mmol/L) [women]
  - TG ≥ 150 mg/dL (1.7 mmol/L) and ≤ 400 mg/dL (4.5 mmol/L)

- For patients entering the trial on a statin ± ezetimibe, the equivalent lipid criteria must be met as follows:

  - the upper limit for LDL-C is adjusted according to the specific statin (± ezetimibe 10 mg) and statin-dose in the table below
  - HDL-C of ≤ 42 mg/dL (1.1 mmol/L) [men] or ≤ 53 mg/dL (1.4 mmol/L) [women]
  - TG ≥ 100 mg/dL (1.1 mmol/L) and ≤ 400 mg/dL (4.5 mmol/L)

  No patient currently receiving a statin ± ezetimibe will be required to discontinue their statin or ezetimibe therapy prior to obtaining baseline laboratory tests or beginning the open-label run-in. All other drugs affecting lipid levels, such as fibrates, niacin, bile acid sequestrants, fish oils, or combination therapy drugs (e.g., niacin extended-release/lovastatin [Advicor®] must be washed out for at least 4 weeks prior to the baseline. Statins and/or ezetimibe are not required to be washed out.

  In eligible patients who are receiving a statin ± ezetimibe at enrollment, the LDL-C upper limit for qualification will be modified as follows:

mg/dL	Statin only					Statin plus Ezetimibe†			
Statin	10 mg	20 mg	40 mg	80 mg		10 mg	20 mg	40 mg	80 mg
None	≤180	180	180	180		≤180	180	180	180
Atorvastatin	≤113	101	92	87		≤88	76	67	62
Pravastatin*	≤141	129	117	110		≤116	104	92	85
Simvastatin	≤129	117	110	97		≤104	92	85	72
Fluvastatin	--	141	135	115		--	116	110	90
Rosuvastatin**	≤97	87	81	--		≤72	62	56	--
SI Units : mmol/L									

Statin	10 mg	20 mg	40 mg	80 mg		10 mg	20 mg	40 mg	80 mg
None	≤4.65	4.65	4.65	4.65		≤4.65	4.65	4.65	4.65
Atorvastatin	≤2.92	2.61	2.38	2.25		≤2.28	1.97	1.73	1.60
Pravastatin*	≤3.65	3.34	3.03	2.84		≤3.00	2.69	2.38	2.20
Simvastatin	≤3.34	3.03	2.84	2.51		≤2.69	2.38	2.20	1.86
Fluvastatin	--	3.65	3.49	2.97		--	3.00	2.84	2.33
Rosuvastatin**	≤2.51	2.25	2.09	--		≤1.86	1.60	1.45	--

* Or lovastatin. ** For rosuvastatin 5 mg, use 113 mg/dL = 2.92 mmol/L. †If on ezetimibe only, use 155 mg/dL

For patients and sites participating in the Magnetic Resonance Imaging (MRI) and HDL proteomics substudies (see Appendices 5 and 6) after August 1, 2009:

c. Willing to participate in the MRI and HDL proteomics substudies.

4.4.2. Exclusion (Non-Inclusion) Criteria:

- Hospitalization for acute coronary syndrome and discharge within 4 weeks prior to planned enrollment (run-in phase)
- Coronary Artery Bypass Graft (CABG) surgery within 1 year of planned enrollment (run-in phase), unless there has been a new, intercurrent acute coronary syndrome event or recurrent angina, associated with angiographic evidence of disease progression (≥ 50% stenosis) in 1 or more native vessels or bypass grafts, regardless of whether subsequently treated with PCI/stenting
- Planned percutaneous coronary intervention (PCI) within 4 weeks prior to planned enrollment (run-in phase)
- Stroke within 8 weeks prior to planned enrollment (run-in phase)
- Fasting glucose >180 mg/dL (10 mmol/L) or hemoglobin A1C >9.0%
- Inability or refusal to use a glucometer for home monitoring of glucose
- CHD associated with unstable angina and symptoms refractory to maximal medical therapy (i.e., persistent Canadian Cardiovascular Society [CCS] Class IV)
- Post-MI course complicated by persistent rest angina, shock, or persistent congestive heart failure (CHF), etc., or if the need/likelihood of urgent revascularization is high
- Patients with left main coronary disease ≥50% and no prior CABG
- Ejection fraction <30%
- Cardiogenic shock, pulmonary edema or CHF unresponsive to standard medical therapy
- Concomitant valvular heart disease likely to require surgery or adversely affect prognosis during follow-up period
- Congenital or primary cardiomyopathy likely to adversely affect prognosis during follow-up period

- Resuscitated out-of-hospital sudden death or symptomatic sustained or non-sustained ventricular tachycardia without an implantable cardioverter-defibrillator (ICD)
- Significant systemic hypertension (blood pressure >200/100 mmHg) unresponsive to medical therapy
- Active peptic ulcer disease
- AST or ALT > 2 times upper limit of normal or active liver disease
- Recent history of acute gout. (For patients with baseline uric acid > 7.0 mg/dL [415 umol/L], treatment with allopurinol is recommended but not mandated)
- Chronic renal insufficiency with creatinine ≥ 2.5mg/dL (220 umol/L)
- Patients who cannot discontinue the following excluded concomitant medications:
    1. Drugs with a high probability of increasing the risk for hepatotoxicity or myopathy, such as those predominantly metabolized by cytochrome P450system 3A4, including, but not limited to: cyclosporine, gemfibrozil, fenofibrate, itraconazole, ketoconazole, HIV protease inhibitors, nefazodone, verapamil, amiodarone
    2. Lipid-lowering drugs (other than the investigational drugs), such as statins, bile-acid sequestrants, fish oils, cholesterol absorption inhibitors (e.g., ezetimibe, *but see section 4.5 on Treatment Protocol for use of ezetimibe to achieve treatment goals)*, fibrates
    3. High-dose, antioxidant vitamins (vitamins C, E, or beta-carotene) that can interfere with the HDL-raising effect of niacin
- Pregnant (or likely to become pregnant) women or pre-menopausal women not using adequate contraception
- Significant co-morbidity likely to cause death in the 3-5 year follow-up period
- Patients with AIDS/active HIV infection, due to potential confounding drug interactions
- Significant active history of substance abuse within the previous 5 years
- Unwillingness/inability to give informed consent or follow study protocol
- Current participation in another clinical study or trial that involves a study drug or intervention
- Unwillingness of patient's physician to allow participation in the study

For patients and sites participating in the MRI and HDL proteomics substudies (see Appendices 5 and 6) after August 1, 2009:

- Bilateral carotid endartectomy
- Weight greater than 250 lbs (113 kg)
- Claustrophobia preventing satisfactory completion of MRI scan
- Glomerular Filtration Rate (GFR) < 60 ml/min/1.73m^2, or ≥ 50% drop in GFR from previous GFR testing point
- Presence of surgical implant or stent incompatible with MRI safety

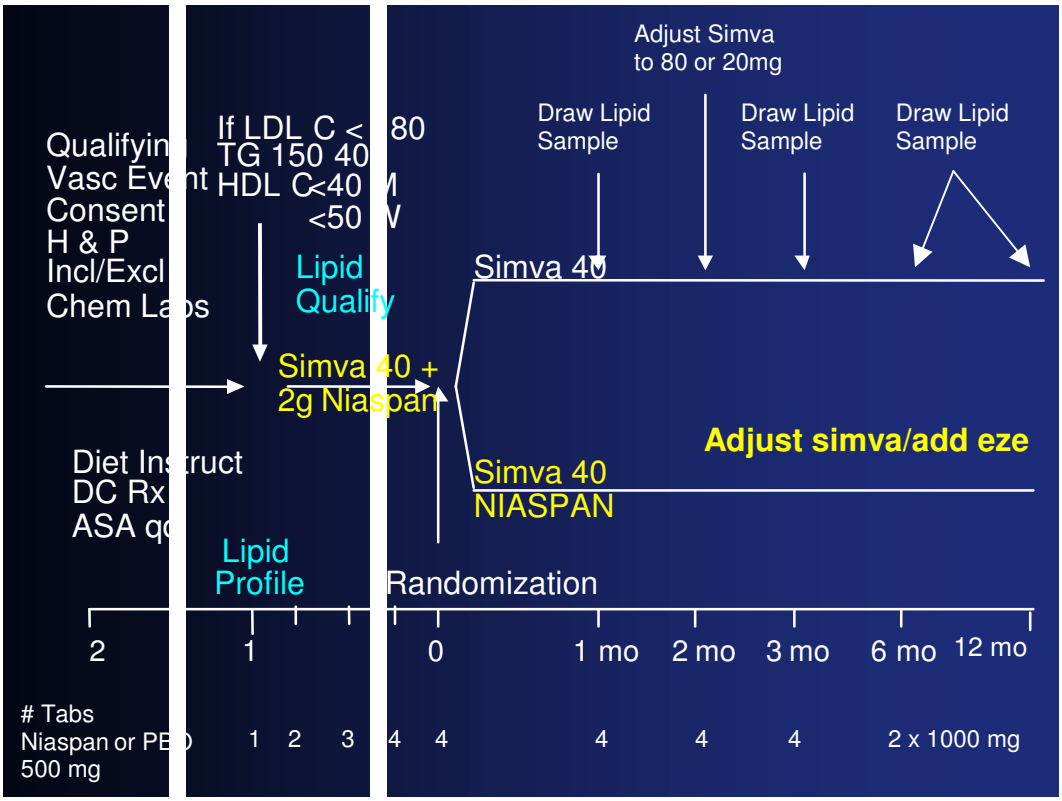
- Any other condition, which, in the opinion of the local physician (radiologist), would contra-indicate MR imaging. (See sample MR Procedure Screening Form - Appendix 5)

4.5 Treatment Protocol (See Study Flow Chart below and in Section 4.9. Also see 4.5.1 below, Treatment Protocol after Discontinuing the Double-Blind Therapy Portion of the Trial)

- The study drugs will be supplied by Abbott, Abbott Park, IL
  - Simvastatin: 10mg, 20 mg, 40 mg and 80 mg tablets
  - Extended-release niacin: 500mg and 1000mg tablets and matching placebos containing 50mg immediate-release niacin
  - Ezetimibe 10 mg
- The maximum doses permitted in this trial are:
  - Extended-release niacin 2000 mg
  - Simvastatin 80 mg
  - Ezetimibe 10 mg

See Manual of Operations for further details on dosing.

Written informed consent must be obtained before any study-specific procedure takes place. Participation in the study and date of informed consent given by the subject should be documented appropriately in the subject's files.



Conversion factors, mg/dL to mmol/L: cholesterol, multiply by 0.0259; for triglyceride, multiply by 0.0113. Entry lipid levels to qualify are modified as described above in 4.4.1.2 for patients on a statin ± ezetimibe.

- Baseline blood sampling for fasting blood glucose, hemoglobin A1C, thyroid function tests (e.g., TSH), liver function tests (ALT, AST) and other relevant blood chemistries will be obtained and monitored at periodic intervals, as deemed appropriate. (See Appendix 4, page 53.)

- Blood samples for frozen storage will be obtained at baseline and at specified points during therapy.

- After patients are deemed to have met inclusion and non-inclusion criteria and informed consent has been obtained, eligible subjects will first undergo an unblinded 4-week run-in period during which they will receive extended-release niacin once-daily in the evening, titrated by 500 mg daily at weekly intervals, beginning with 500 mg once-daily in the evening to a maximum of 2000 mg, together with simvastatin 40 mg, to establish tolerability of this combination. Administration of aspirin 325 mg up to 30 minutes prior to dosing will be encouraged. ***In order to proceed to randomization, a patient must tolerate a minimum of 1500 mg extended-release niacin.*** The titration period may be extended up to 8 weeks in order to establish tolerability. (See Manual of Operations for further detail.)

- Patients who successfully complete the unblinded, open-label run-in period and tolerate this combination therapy will be then be randomized to receive study medication once-daily with either statin monotherapy, beginning with simvastatin 40 mg, or combination therapy with niacin extended-release /simvastatin at a dose of 2000/40 (or 1500/40, if 1500 mg was the highest tolerated dose of extended-release niacin during the run in), for 8 weeks. After 8 weeks (2 months), the dose of simvastatin will be increased to 80 mg for patients in either treatment group if LDL-C is > 80 mg/dL (2.1 mmol/L) based on a sample drawn at 1 month. If LDL-C is < 40 mg/dL (1.0 mmol/L), the dose of simvastatin will be decreased to 20 mg. Values for LDL-C will be provided to the investigators throughout the trial; however, values for other lipid parameters such as HDL-C and triglycerides will *not* be provided.

  At 12 weeks (3 months), a fasting lipid sample will also be drawn. If a patient's LDL-C is > 80 mg/dL (2.1 mmol/L), the patient should be contacted to come into the clinic. If he or she is currently taking simvastatin 40 mg, the dose of simvastatin should be increased to 80 mg. If he or she is currently taking simvastatin 80 mg, then ezetimibe 10 mg should be added to their treatment regimen. ***In patients who are given ezetimibe at this point, the dose of simvastatin should be simultaneously decreased back to 40 mg. In either case, changes to the treatment regimen should be done no later than 16 weeks (4***

months).

At 24 weeks (6 months), fasting lipid samples will again be obtained. At this visit, the following titrations/dose adjustments should be made, preferably in the clinic at or just before 9 months, in lieu of the 9-month telephone contact (see Table page 34), since it may be necessary to dispense additional or different simvastatin tablets, or ezetimibe.

- If the LDL-C is > 80 mg/dL (2.1 mmol/L) but < 100 mg/dL (2.6 mmol/L), and the patient is receiving 40 mg of simvastatin and 10 mg of ezetimibe, no adjustment in the dosage of either drug will be made based on the LDL-C result.
- If LDL-C is > 80 mg/dL (2.1 mmol/L) but < 100 mg/dL (2.6 mmol/L), and the patient had been receiving only simvastatin 40 mg, the dose of simvastatin should be doubled to 80 mg.
- If LDL-C is > 80 mg/dL (2.1 mmol/L) but < 100 mg/dL (2.6 mmol/L), and the patient had been receiving simvastatin 80 mg, then ezetimibe 10 mg should be added to their treatment regimen. ***In patients who are given ezetimibe at this time, and the dose of simvastatin had been 80 mg, the dose of simvastatin should be simultaneously decreased back to 40 mg.***

- Only in patients whose LDL-C remains $\geq$ 100 mg/dL despite therapy with simvastatin 40 mg and ezetimibe 10 mg, may the dose of simvastatin be increased to 80 mg in combination with ezetimibe.

At 12 months and at 36 months (and, as an option for patients with >5 years of follow up, at 60 months), fasting lipid samples will again be obtained. However, the only simvastatin/ezetimibe dose adjustments permitted, based on these sample results, will be for LDL-C > 100 mg/dL [2.6 mmol/L] or < 40 mg/dL [1.0 mmol/L]. See Manual of Operations for further details about dose titration and addition of ezetimibe.

Dose adjustment of both simvastatin, niacin extended-release, and ezetimibe is also allowed throughout the trial, as needed, to manage possible adverse events such as muscle aches or weakness, marked fatigue, nausea, or intolerable flushing, as described in the Manual of Operations

Because of the possibility that cutaneous flushing in the combination therapy arm could potentially unmask the identity of blinded therapy to both patients and study personnel, each placebo tablet for extended-release niacin will include a small, sub-therapeutic dose of crystalline (immediate-release) niacin 50 mg.

- All patients will be encouraged to take aspirin 325 mg (or ibuprofen or other non-steroidal anti-inflammatory) up to 30 minutes prior to dosing with the investigational drug to alleviate flushing, to take the investigational drug with a

lowfat snack at bedtime, and to avoid hot or spicy food/drink around the time of dosing.

- Excluded concomitant medications

  - Drugs with a high probability of increasing the risk for hepatotoxicity or myopathy, such as those predominantly metabolized by cytochrome P450 system 3A4, including: cyclosporin, gemfibrozil, fenofibrate, itraconazole, ketoconazole, HIV protease inhibitors, nefazodone, verapamil, amiodarone

  - Lipid-lowering drugs (other than the investigational drugs), such as statins, bile-acid sequestrants, fish oils, cholesterol absorption inhibitors (e.g., ezetimibe, except for its use as described above to achieve study protocol treatment goals for LDL-C), fibrates

- Treatment adherence

  It is recommended that, unless clear contraindications arise, patients be strongly encouraged to adhere to their treatment regimen with the study drugs for the duration of the trial. Patients should be counseled in particular about the possibility of flushing and ways in which to manage or mitigate it. Any interruptions of therapy should, if possible, be brief (e.g., < 4 weeks) and for only for clinically indicated reasons, such as adverse events. Discontinuations will be discouraged as much as possible. Any discontinuations should be based on compelling clinical reasons. For every patient, an assessment of study drug adherence must be obtained at each scheduled visit.

4.5.1 Treatment Protocol after Discontinuing Double-Blind Therapy with Extended-Release Niacin or Placebo (Amendment 6, Revision June 1, 2011)

All participants will discontinue their double-blind therapy with extended-release niacin or placebo on May 25, 2011. This change is made in reponse to the recommendation of the DSMB, which the NHBLI accepted on May 4, 2011. During an extension to follow-up (18 months after the end of the randomized trial), the patient's personal physician will be responsible for lipid management. The study will provide no study drug or intervention during this follow-up period.

4.6 Assessment of Clinical Events (Revised April 6, 2010. See also 4.6.6 Assessment of Clinical Events After Stopping Double-Blind Therapy below on page 31)

All events occurring between randomization and the study end date (inclusive) must be recorded. Only adjudicated events will be included in the final analyses. Further details on the assessment of clinical events and their definitions will be found in the Clinical Events Committee charter.

4.6.1 Primary Efficacy Endpoint (E5)

The first occurrence of any of the following major adverse cardiovascular events, as validated by the CEC in concert with the core ECG laboratory:

- CHD death
- Non-fatal MI (including silent MI)
- Ischemic stroke
- Hospitalization for acute coronary syndrome
- Symptom-driven coronary or cerebral revascularization

4.6.2 Definitions of components of the primary efficacy endpoint

4.6.2.1 Coronary Heart Disease Death

Defined as any death with a clear relationship to underlying coronary heart disease (including death secondary to acute MI, sudden death, unobserved and unexpected death, and other death not definitely attributed to a nonvascular cause).

4.6.2.2 Myocardial infarction (MI)

Based on ACC definitions for measuring outcomes[46], the following situations will be considered:

- For patients with no recent cardiac intervention within 72 hours, at least one of the following must be present :
  CK-MB elevation ≥2 times upper limit of normal (ULN)
  Troponin elevation ≥2 times ULN

  With at least one of the following:
  - Ischemic symptoms within 48 hours
  - New ST depression ≥0.5mm in 2 contiguous leads or T wave inversion ≥1mm in leads with predominant R wave or R/S ratio > 1.0 in 2 contiguous leads
  - LBBB (new)
  - ST elevation (new ST elevation in at least 2 contiguous leads ≥0.2mV in V1, V2, or V3 or ≥0.1mV in other leads)
  - New R wave ≥ 40 ms in V1, V2 with R/S ≥ 1 in V1 and with concordant positive T-wave in the absence of a conduction defect or Q wave ≥20ms or QS complex in leads V2 and V3.
  - New Q waves ≥30 ms in 2 contiguous leads
  - Imaging evidence of loss of viable myocardium
- Patient who underwent recent PCI/CABG (within 72 hours)
  - PCI: CK-MB ≥3 times ULN or development of new Q wave as defined above or troponin ≥5 times ULN
  - CABG: either CK-MB ≥5 x ULN and new Q waves, or CK-MB≥10 times ULN (with or without Q wave) or troponin ≥20 times ULN

Silent MI detected on routine ECG will be included in the definition of MI.

Amendment 6, Revision June 1, 2011

One final ECG will be obtained approximately 18 months following discontinuation of double-blind study drug to detect any silent MI that occurrs during this follow-up period.

4.6.2.3 Stroke

Defined as an acute neurological vascular event with focal signs lasting more than 24 hours *and* considered to be of ischemic origin. If a previous deficit has worsened, it must have lasted more than one week, or more than 24 hours if accompanied by an appropriate new CT or MRI finding.

CT scan or MRI should be performed and provided to the CEC to allow exclusion of non-vascular causes.

4.6.2.4 Hospitalization for Acute Coronary Syndrome

a. High-risk ACS hospitalization

Hospitalization is defined as admission to hospital or emergency room stay that exceeds 23 hrs. High risk ACS is defined as a history of accelerating tempo of ischemic symptoms in the prior 48 hrs or prolonged (at least 20 min) ongoing rest pain presumed to be ischemic AND new ECG evidence of myocardial ischemia as defined above or elevated CKMB/troponin with at least 1 sample above upper normal limits but < 2 times ULN and a characteristic rise or fall of the biomarker or hemodynamic compromise.

b. Not high-risk ACS hospitalization

Admission for worsening ischemic chest pain or chest pain equivalent and:

- ECG changes that do not meet AIM HIGH ECG high risk criteria and negative biomarkers
- Or evidence for myocardial ischemia on non-invasive testing
- Or coronary disease progression on angiography not due to restenosis
- Or residual incomplete coronary revascularization on prior angiography

4.6.2.5 Symptom-Driven Coronary or Cerebral Revascularization

- Symptoms lead to revascularization regardless of whether or not the procedure itself is successful
- Worsening symptoms after randomization associated with ischemia demonstrated on non-invasive testing or coronary disease progression at angiography followed by PCI or CABG at least 30 days after randomization

- Worsening symptoms after randomization associated with revascularization of the cerebrovascular system at least 30 days after randomization
- Revascularization procedures for restenosis, early or late stent thrombosis will be recorded but not counted as a primary endpoint
- Elective coronary revascularization procedures in non-symptom driven patients will be recorded but not counted as a primary endpoint even if non-invasive testing is abnormal since it cannot be determined if this represents disease that was present before randomization (for example, as part of a non-cardiac preoperative work-up, a non-invasive test reveals ischemia leading to angiography and coronary revascularization)

Revascularization is defined as any of the following procedures:

- Coronary revascularization: PCI (includes percutaneous transluminal coronary angioplasty [PTCA], coronary stenting, and others such as brachytherapy, atherectomy, laser, and rotational ablation) or CABG.
- Cerebrovascular revascularization: carotid endarterectomy, carotid percutaneous transluminal angioplasty (with or without stent).

4.6.3 Secondary Efficacy Endpoints

- To evaluate the effect of therapy on the composite endpoint of CHD death, nonfatal MI, hospitalization for high risk ACS, or ischemic stroke (E4)

- To evaluate the effect of therapy on the composite endpoint of CHD death, non-fatal MI, or ischemic stroke (E3)

- To evaluate the effect of therapy on cardiovascular mortality

4.6.4 Tertiary Efficacy Endpoints

- To evaluate the effect of therapy on total mortality

- To evaluate the effect of therapy on the composite endpoint of, and the individual components and subcomponents of the composite endpoint of death, non-fatal MI, stroke, hospitalization for acute coronary syndrome, or any arterial revascularization

- To evaluate the effect of therapy for preventing clinical events, as defined above, among patients meeting current criteria for metabolic syndrome as defined by the NCEP ATP III, or future criteria for metabolic syndrome as they may evolve, or diabetes

- To assess the effects of statin monotherapy versus combination therapy on lipids and lipoproteins, including apoA-I, apoB, apoC-III, Lp(a), HDL

subfractions/particle size, LDL size and subclass distribution, and their relationship to outcome

- To assess the effects of therapy on inflammatory markers, such as C-reactive protein and fibrinogen, and their relationship to outcome

4.6.5 Definitions of components of secondary and tertiary efficacy endpoints

- 4.6.5.1 Hospitalization

Defined as at least one overnight stay (or admission to emergency room $\geq$ 23 hours)

- 4.6.5.2 Cardiovascular Mortality

Defined as death from coronary heart disease (defined above), ischemic stroke or death as a result of a symptom-driven vascular procedure. When the cause of death is stroke and it cannot be determined if the event is primary ischemic or hemorrhagic, the event will be categorized as an ischemic stroke. Deaths that result from rupture of an abdominal aortic aneurysm or surgery for aneurysm will not be counted as a cardiovascular death.

4.6.2 Assessment of Clinical Events After Stopping Double-Blind Therapy (Amendment 6, Revision June 1, 2011)

The primary endpoint for the 18 month follow-up period after stopping double-blind therapy with extended-release niacin or placebo will remain the same: CHD death, nonfatal MI, ischemic stroke, hospitalization for ACS, or symptom-driven revascularization. The CEC will continue to adjudicate all such events.,.

4.7. Patient safety

4.7.1 Adverse events

An adverse event is any untoward medical occurrence in a patient or clinical investigation patient administered a pharmaceutical product and which does not necessarily have to have causal relationship with this treatment.

All adverse events, regardless of seriousness or relationship to study drug, are to be recorded on the Case Report Form devoted to recording adverse events. Whenever possible, symptoms should be grouped as a single syndrome or diagnosis. The investigator should specify the date of onset, maximal intensity, action taken with respect to study drug, corrective therapy given, outcome and his/her opinion as to whether there is a reasonable possibility that the adverse events was caused by the study drug.

4.7.2 Serious adverse events

Serious adverse event is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a medically important event

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

In the case of a serious adverse event, the investigator must immediately fax the signed and dated case report form, accompanied with photocopy of all examinations, to the representative of the monitoring team.

4.7.3 Follow up of adverse events and serious adverse events

The investigator should take all appropriate measures to ensure the safety of the patients. The outcome of any adverse events (clinical signs, laboratory values or others) should be followed up until they return to normal or until stabilization of the patient's condition.

4.8 Patient Withdrawal

4.8.1 Withdrawal criteria

Occurrence of an outcome event according to the judgment of the investigator is not considered as a reason for study drug discontinuation. Permanent study drug discontinuation is only clearly justified for an adverse event or when a patient or his or her physician insists on withdrawing from study drug treatment, generally for clinical reasons. Study drug discontinuation should be avoided as far as possible. The reason for study drug discontinuation will be recorded on the Case Report Form.

4.8.2 Reasons for Withdrawal

The patients may withdraw from the study drug at any time and for any reason, or this may be at the investigator's discretion. (See above.) In any case, follow-up for efficacy and safety endpoints should be continued.

4.8.3 Follow-up After Withdrawal

- Patients who prematurely discontinue study drug are not to be replaced
- All randomized patients must be followed up according to the study flowchart until study end date or death, regardless of whether they discontinue study drug prematurely or not. Any event occurring after early study drug discontinuation will be recorded up through the study end date.
- In order to follow the medical status of the patients, especially when they withdraw after having experienced an adverse event, investigators are encouraged to obtain information from the patient's primary care practitioner (physician or any other medical care provider). Investigators are also requested to try as much as possible to re-contact those patients at the end of the trial to obtain at least their vital status as well as their stroke or MI status, and thus avoid lost to follow-up for the efficacy assessment.
- If patients are lost to follow-up, the Case Report Form must be completed up to the last visit or contact.

4.9 Study Procedures

4.9.1 Visit schedule (See Flow Chart below)

4.9.2 Screening and run-in procedures

Potentially eligible patients will be identified from all relevant in-patient and out-patient sources, including coronary care units, stroke centers, invasive and noninvasive laboratories, office practices and specialty clinics. Patients referred

from other physicians or other sources will also be screened. The patient will receive complete information about the study both orally and in writing. Written informed consent must be obtained prior to performing any study related procedures, including phlebotomy to obtain screening laboratories, withdrawal of current lipid-modifying drugs, electrocardiograms, chest X-rays, etc.

As described above, eligible subjects will first undergo an unblinded 4-week run-in period to establish tolerability of the combination therapy. This will ensure a higher likelihood of long-term adherence, once randomization to the double-blind phase of the trial is initiated. Key baseline patient characteristics will be recorded in the Case Report Form. Eligible subjects will receive extended-release niacin once-daily in the evening, titrated by 500 mg daily at weekly intervals, beginning with 500 mg once-daily in the evening to a maximum of 2000 mg, together with simvastatin 40 mg, to establish tolerability of this combination. Administration of aspirin 325 mg up to 30 minutes prior to dosing will be encouraged. In order to proceed to randomization, a patient must tolerate a minimum of 1500 mg extended-release niacin. The titration period may be extended up to 8 weeks, if necessary, to establish tolerability. (See Manual of Operations.) The number of patients failing to proceed to randomization, and the reason(s) why, must be documented.

4.9.3 Randomization

After successful completion of the unblinded, open-label run-in period with the combination therapy, patients will then be randomized to receive blinded study medication with either simvastatin monotherapy, or combination therapy (simvastatin plus niacin extended-release). Randomization will be stratified by site, gender and history of diabetes. All patients who are randomized will be included in the intent-to-treat analyses, whether or not they are subsequently found to be eligible or actually receive the allocated treatment. All randomized patients will be followed until the study end date or death. Study drug administration should be initiated as soon as possible after randomization.

4.9.4 Clinical follow-up visits (See Flow Chart below and Manual of Operations. Also see Clinical Follow-Up Visits After Stopping Double-Blind Therapy with Extended-Release Niacin or Placebo, below on page 37)

4.9.4.1 Screening, baseline visit and run-in period

- Baseline visit:
  - Demographic information, medical history, physical examination, current medical treatments, and ECG
  - Fasting blood lipids and lipoproteins (See study flow chart on page 34 and Appendix 4, page 53)

- Fasting blood glucose, hemoglobin A1C, thyroid function tests, uric acid, CK, liver function tests (See study flow chart on page 34 and Appendix 4, page 53)
- Blood samples for frozen storage
- Other laboratory tests, as described in Appendix 4 and Manual of Operations
- Baseline EQ5D health outcome questionnaire.

For patients and sites participating in the MRI and HDL proteomics substudies (see Appendices 5 and 6) after August 1, 2009:

- Creatinine for calculation of GFR, obtained within 4 weeks prior to date of planned baseline MRI
- Blood for HDL proteomics substudy (SPECIAL COLLECTION, PROCESSING, and SHIPPING PROCEDURES REQUIRED).

- Run-in period:
  - Adverse events occurring during the run-in period and adherence with study drug(s) will be recorded at –2 weeks (by telephone) and at the end of the run-in

4.9.4.2 Randomization (Day 0)

Patients tolerating the combination therapy during the run-in period will be randomized. All patients will receive simvastatin open-label. Patients will be randomized to blinded therapy with either extended-release niacin or placebo matching extended-release niacin.

For patients and sites participating in the MRI and HDL proteomics substudies (see Appendices 5 and 6) after August 1, 2009:

Schedule baseline MRI scan. The baseline MRI scan must fall within 13 weeks of randomization and within 4 weeks of a creatinine determination.

4.9.4.3 Follow-up (See Flow Chart on page below and Appendix 4)

- 1-month follow-up visit (Day 30 ± 7)
  - Fasting blood sample for lipids
  - Fasting blood sample for chemistries
  - Record of efficacy endpoints, if any
  - Record of adverse events, if any
  - Study drug adherence
  - Record of interventions, if any

- 2-month follow-up visit (Day 60 ± 7)
  - Increase or decrease dose of simvastatin as needed, based on achieving LDL-C target $\leq$ 80 mg/dL (2.1 mmol/L) but $\geq$ 40 mg/dL (1.0 mmol/L)
  - Record of efficacy endpoints, if any
  - Record of adverse events, if any

- Study drug adherence
- Record of interventions, if any

- 3-month follow-up visit (Day 90 ± 10)
  - Fasting blood sample for lipids
  - Fasting blood sample for chemistries
  - Record of efficacy endpoints, if any
  - Record of adverse events, if any
  - Study drug adherence
  - Record of interventions, if any
  - ***If required, within 1 month of this visit, increase or decrease dose of simvastatin and/or add ezetimibe 10 mg, as described above in section 4.5***

- 6-month follow-up visit (Day 180 ± 10)
  - Fasting blood sample for lipids
  - Fasting blood sample for chemistries
  - Record of efficacy endpoints, if any
  - Record of adverse events, if any
  - Study drug adherence
  - Record of interventions, if any
  - ***If required, patient returns at 9 months to increase or decrease dose of simvastatin and/or add ezetimibe 10 mg, as described above in section 4.5***

- Subsequent 6-month visits (every 180 Days ± 10)
  - Fasting blood determinations for lipids and chemistries, as per protocol (Appendix 4)
  - Record of efficacy endpoints, if any
  - Record of adverse events, if any
  - Study drug adherence
  - Record of interventions, if any
  - Electrocardiograms will be obtained at year 1 and annually thereafter
  - ***At 12-months and again at 36 months (and, as an option for patients with >5 years of follow up, at 60 months), dose adjustments for simvastatin/ezetimibe will be permitted, but only for LDL-C > 100 mg/dL [2.6 mmol/L] or < 40mg/dL [1.0 mg/dL]***
  -EQ5D at annual follow-up visits

- Telephone follow-up contact with patient at 2 weeks (±4 days), 36 weeks (±7 days), and, thereafter, every 24 weeks (6 months, ±10 days)
  - Screen for possible efficacy endpoints or adverse events
  - Patients will be asked to return to the clinic to assess for any endpoints or events identified

- Final follow-up visit (study end date): visit may occur within 30 days after the study end date; however, only events occurring up to and including the scheduled actual study end date will be included in the primary efficacy analysis

- Record of efficacy endpoints, if any
- Record of adverse events, if any
- Study drug adherence
- Record of interventions, if any
- Electrocardiogram

Every attempt should be made to complete the follow-up visits during the defined window periods. A final follow-up visit is required for all patients. In the rare cases a final follow-up visit cannot occur within the 30-day timeframe following study end date, any attempt to contact the patient must be recorded on a special contact form, until/unless appropriate information is obtained.

For patients and sites participating in the MRI and HDL proteomics substudies (see Appendices 5 and 6) after August 1, 2009:

Follow-up MRI scans at year 1 and year 2. See Manual of Operations for details. Note that GRF must be recalculated within 4 weeks prior to each follow-up scan. The follow up scans at years 1 and 2 must fall within 4 weeks of the coinciding visits in the main trial.

Blood for HDL proteomics substudy at year 1 and year 2.

4.9.5 Clinical Follow-Up Visits After Stopping Double-Blind Therapy Portion of Trial (Amendment 6, Revision June 1, 2011)

- Visit for end of double-blind treatment period, All patients will be seen in the clinic between June 1, 2011 and August 15, 2011 as an end to the double-blind treatment phase. At that visit, the following will be obtained:

- Fasting blood sample for lipids
- Fasting blood sample for AST/ALT and glucose
- ECG if none obtained within the past 6 months
- Record of efficacy endpoints, if any
- Record of adverse events, if any
- Concomitant medications
- Record of revascularizations, if any
- Transition management of lipid therapy (statins and other medications as appropriate) to participant's personal physician

- Follow-up after Double-Blind Treatment Phase:
- At approximately 4 months after the visit for the end of the double-blind treatment period of trial visit, participants will be sent a standardized reminder about participation in extension to this portion of follow-up.

- At 9 months (± 1 month) after the visit for the end of the double-blind treatment period of trial visit a telephone contact visit will occur

- Record current lipid therapies
– Query for possible efficacy endpoints, revascularizations or specific cardiovascular adverse events and hospitalizations.

- At approximately 13 months after the visit for the end of the double-blind treatment period of trial visit, participants will be sent a second standardized reminder about participation in extension to follow-up.

- At 18 months (± 1 month) after the visit for the end of the double-blind treatment period of trial visit, the patient will be seen in-clinic for a final visit. At this visit, the following will be obtained:

- Record current lipid therapies
- Fasting blood sample for lipids
- Fasting blood sample for AST/ALT and glucose
- Obtain a 12-lead ECG for ascertainment of possible silent MI or persistent atrial fibrillation
- Record of efficacy endpoints, if any
- Record of specific cardiovascular adverse events or hospitalizations, if any
- Concomitant medications
- Record of revascularizations, if any

VISIT‡	Baseline visit or prior	-2 wk phone FU		2 wk phone FU	1mo visit FU	2mo visit FU	3mo visit FU	6mo visit FU	9mo phone FU	12mo visit FU	15mo phone FU	18mo visit FU	21mo phone FU	24mo visit FU	27mo phone FU	30mo visit FU	33mo phone FU	36mo visit & q6 mo visits to study end	39 mo visit & q6 mo visits to study end	Final visit FU
Day	Run-in period		D 0		D 30±7	D 60±7	D 90±10	D 180±10		D 360±10		D 540±10		D 720±10		D 900±10		D1080±10, etc (q6 mo)	D1170±10 etc (q6 mo)	From study end date to 30 days after
Medical history	x																			
Previous medications	x																			
Inclusion/exclusion criteria	x																			
Informed consent/patient demography	x																			
Vital signs	x		x		x	x	x	X		x		x		x		x		x		x
ECG (annually)*	x									x				x				x		x
Lab tests (blood lipids, glucose, etc)** - see lab chart Appendix 4	x				x		x	X		x	(x)	x		x		x		x	(x)	x
Randomization			x																	
Study drug allocation	x		x		x	x	x	X		x		x		x		x		x		
Adherence		x	x		x	x	x	X	x	x	x	x	x	x	x	x	x	x	x	
Primary efficacy endpoints				x	x	x	x	X	x	x	x	x	x	x	x	x	x	x	X	x
Adverse events		X	x	x	x	x	x	X	x	x	x	x	x	x	x	x	x	x	x	x
EQ5D***	x									x				x				X***		
MRI scan†			x							x				x						
†Blood for HDL proteomics substudy	x									x				x						

See Table footnotes on next page.

*ECG at baseline and annually **See separate schedule of laboratory tests in Appendix 4 *** At baseline & annually

†For patients/sites participating in the MRI and HDL proteomics substudies (see Appendices 5 and 6) after August 1, 2009.

FU = follow up D = Day (X) = check AST in clinic if dose adjustments made previously at months12 or 36

VISIT‡	Post double-blind tx period: 9 mo	Post double-blind tx period: 18 mo
Day	9 mo (± 1 mo) after end of blinded treatment visit	9 mo (± 1 mo) after end of blinded treatment visit
Medical history		
Previous medications		
Inclusion/exclusion criteria		
Informed consent/patient demography		
Vital signs		
ECG (annually)*		X
Lab tests (blood lipids, glucose, etc)** - see lab chart Appendix 4		X
Randomization		
Study drug allocation		
Adherence		
Primary efficacy endpoints	X	X
Adverse events		
EQ5D***		
MRI scan†		

†Blood for HDL proteomics substudy		

5. Statistical Considerations (See Sections 4.6 , Assessment of Clinical Events, above, and 5.5, Protocol Modification, below)

5.1 General Statistical Approach

AIM-HIGH is a randomized, multi-center controlled clinical trial in patients with established vascular disease and atherogenic dyslipidemia. Patients will be randomized in a 1:1 proportion to a combination of simvastatin and extended-release niacin or simvastatin alone. General issues concerning the statistical analyses are:

- Primary and secondary efficacy analyses will be performed under the principle of intention-to-treat.
- Safety and some exploratory secondary analyses will be restricted to treated patients.
- All statistical analyses of efficacy outcomes will be performed at the 2.5% significance level using 1-sided tests
- Primary and secondary efficacy endpoints will be analyzed using proportional hazards survival analysis techniques and a Wald Chi-square statistic to test the difference between the two treatment groups
- A formal interim analysis plan will be drafted and approved prior to the start of the trial. Group sequential methods will be used to monitor the trial for efficacy and harm.

Additional details concerning the final and interim analysis plans are given below. Further details will be provided in a detailed statistical analysis plan that will be finalized prior to any analysis of trial data.

5.2 Outcome Parameters and Analysis Datasets

The primary outcome (E5) is the time from randomization to the first occurrence of any of the following events: coronary heart disease death, nonfatal myocardial infarction, ischemic stroke, hospitalization for acute coronary syndrome, or symptom-driven coronary or cerebral revascularization.

The secondary outcomes are:

- cardiovascular death, nonfatal MI, high-risk acute coronary syndrome, or ischemic stroke (E4)
- cardiovascular death, nonfatal MI or ischemic stroke (E3)
- cardiovascular mortality

The tertiary outcomes are:

- total mortality
- the composite endpoint of, and the individual components and subcomponents of the composite endpoint of, death, non-fatal MI, stroke, hospitalization for acute coronary syndrome, or any revascularization
- clinical events, as defined above, among patients who meet the current criteria for

metabolic syndrome as defined by the NCEP ATP III, or future criteria for metabolic syndrome as they may evolve, or diabetes
- lipids and lipoproteins, including lipoprotein subclasses, and inflammatory markers (e.g., fibrinogen, hs-CRP), analyzed in terms of therapy effects and clinical outcomes

As noted, the primary efficacy analyses will be performed based on the intention-to-treat principle, including all randomized patients analyzed according to their treatment assignment.

The Statistical Analysis Plan will present additional details concerning the planned analyses.

5.3 Statistical Analyses

Primary Endpoint

AIM-HIGH is designed to compare the time-to-event distributions of the simvastatin versus the simvastatin+niacin treatment groups; the null hypothesis is that the hazard ratio for the two survival distributions is equal to one. The primary test of the null hypothesis will be based on a stratified log-rank test, including gender and history of diabetes as strata and using a one-sided hypothesis with a 0.025 significance level. Cumulative event rate estimates will be used to describe the survival probabilities for each treatment group at pre-specified clinically significant time points. Patients with non-CHD deaths will be censored at the time of death.

An estimate of the hazard ratio, along with a 97.5% confidence interval, will be derived from a Cox proportional hazards model. The assumption of proportional hazards for the treatment group factor will be assessed visually using log-cumulative hazard plots and by including a time-by-treatment interaction in the Cox model. Any indication of departures from the proportional hazards assumption will be investigated more formally and discussed in the presentation of the results.

Secondary endpoints will be examined in a similar manner.

Tertiary analyses may be stratified based on metabolic syndrome status at baseline or other demographic or clinical parameters. Subgroup analyses to explore potential variation in the treatment effect will utilize Cox proportional hazards models including treatment-by-subgroup interaction terms. Significance levels will not be adjusted in the subgroup analyses, as these analyses are exploratory in nature and are to be interpreted descriptively. Further details of the exploratory analyses will be presented in the Statistical Analysis Plan.

5.4 Interim Analyses and Sample Size Adjustment

Interim analyses based on a group sequential design that includes early stopping rules for benefit and futility while preserving the overall Type I error rate (O'Brien-Fleming)[46] will be incorporated into the AIM-HIGH study design. The DSMB will review unblinded

data reflecting the local investigator's assessment of endpoint (i.e., non-adjudicated data) at pre-specified times (for example, every six months). Interim analyses will be performed with significance levels determined using the alpha-spending rule of Lan and Demets.[47] Sequential boundaries will be designed to assess both unexpectedly large benefit or lack of efficacy. Approximately 850 events will be observed during the trial, based on sample size calculation assumptions. The first interim analysis will occur after at least 425 events have been observed, with additional interim analyses triggered by occurrence of a pre-specified total number of events. Specific statistical guidelines for data monitoring will be discussed and formalized in a separate Interim Analysis Plan document.

5.5 Sample Size Determination:

As originally designed, in AIM-HIGH, qualified patients will be enrolled in at least 54 clinical sites over a planned 2 year period; follow-up will be completed with a mean of at least 4 years and a minimum follow up of 3 years. For the current study, the goal is that the study population will comprise 30% women. The lipid inclusion criteria and focus on high-risk vascular disease patients will ensure that the vast majority of patients will have metabolic syndrome (85% of patients in HATS with atherogenic dyslipidemia had metabolic syndrome).

Sample size calculations were based on estimates of untreated 4-year event rates derived from the ongoing CHARISMA trial (personal communication: William E. Boden, MD, member of the Steering Committee), due to the similarities of the patients enrolled in this study and the patient population proposed for AIM-HIGH (i.e., high-risk patients with established vascular disease and lipid abnormalities). CHARISMA examines a secondary endpoint including cardiovascular death, non-fatal MI, stroke and hospitalization for an acute ischemic event; this is the primary endpoint proposed for AIM-HIGH. To date, CHARISMA has observed a 9.13% annual event rate for the secondary endpoint. We assumed that 68% of the CHARISMA population is using a lipid-lowering drug with an associated 30% decrease in annual event rate. This results in an estimated 11.5%/year event rate in untreated patients and an 8.0%/year event rate in patients treated with lipid-lowering drugs. These annual event rates correspond to 4-year event rates of 39% and 28% in the untreated and lipid-lowering groups.

The event rate estimates were further adjusted for presence of metabolic syndrome. Approximately 75% of the CHARISMA population have metabolic syndrome; it is expected that 90% of the AIM-HIGH population will fall into this category and have an associated 60% increased risk. With additional assumptions that there will be a 50% decrease in risk in the niacin + simvastatin group compared to placebo, and that 10% of both treatment groups will stop using all drugs and 10% of the combination treatment group will stop using niacin but continue with simvastatin only, the estimated 4-year AIM-HIGH primary endpoint event rates based on CHARISMA data are 30% in the simvastatin treatment group and 23% in the combination therapy treatment group.

Based on these 4-year estimates and an assumption of exponential survival time,[48] a one-sided test with an alpha-level of 0.025, 2 years for patient accrual and a minimum of 3 years of follow-up, a total sample size of 3,300 patients will result in 99% power to detect a difference between hazard rates of 0.067 and 0.089 (hazard ratio=0.75) in the simvastatin + niacin and simvastatin therapy groups, respectively. With the above assumptions, it is expected that approximately 890 events will be observed during the trial.

AIM-HIGH power estimates based on data from other comparable clinical trials and using similar calculations to those described above are:

Study	CHAR-3	CHAR-4	CHAR-4*	VA-HIT	VA-HIT	HATS	HATS	4S
N=3000	**.64**	**.98**	**.90**	**.85**	**.96**	**.88**	**.99**	**.94**
N=3300	**.68**	**.99**	**.93**	**.88**	**.97**	**.91**	**.996**	**.96**
N=3800	**.74**	**.996**	**.96**	**.92**	**.99**	**.94**	**.999**	**.98**

CHAR: Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance (CHARISMA)[49], and unpublished data (personal communication, CHARISMA Steering Committee).
CHAR* This column assumes that the CHARISMA quadruple composite endpoint, which is currently unadjudicated, is 33% lower after adjudication.
VA-HIT[18]
HATS[19]
4S (Scandanavian Simvastatin Survival Study)[50]

5.6 Protocol Modification

Power estimates were recomputed based on an examination of interim overall (i.e., still blinded to treatment assignment) rates of the originally planned primary endpoint (E4). As a result of the much lower than expected overall event rate, the primary endpoint was redefined, as described above to time to the first of:

- CHD death
- Non-fatal MI (including silent MI)
- Ischemic stroke (fatal or nonfatal)
- Hospitalization for acute coronary syndrome
- Symptom-driven coronary or cerebral revascularization

Actual data, blinded to treatment, was examined to provide an estimate of the rate of this new composite primary endpoint (E5). With 2,783 participants randomized and 3,626 patient-years of exposure, the annualized rate of E5 was estimated at 0.0665.

The differential effect of extended release niacin is expected to begin to be evident 3 months after randomization, with full effect 6 months after randomization. This delayed treatment effect decreases the power of the study under fixed assumptions of accrual, expected treatment effect and number of primary events observed.

At the time of the blinded examination of interim data, the annualized rate of discontinuation of blinded study drug (extended release niacin or placebo) was .075 (1-year Kaplan-Meier estimate 0.083 and 3-year Kaplan Meier estimate 0.071). Given that the 3,300 participants were recruited over a 3.5 year period and that 3% of participants overall would be lost to follow-up and very few would discontinue statin therapy, the following summarizes power calculations and estimates of trial duration. Conservatively, the observed overall event rate was presumed to be an estimate of the control rate (i.e., the null hypothesis was assumed). Adjustments were made to account for the delayed treatment effect, rate of discontinuation of blinded therapy and loss to follow-up. Power, study duration and number of events were estimated using a Markoff model.

- Power, Number of Events and Trial Duration Assuming: Alpha level: 0.025, one sided
- Enrollment of 3,300 participants over 3.5 years (actual)
- Overall 3% (99 participants) lost to follow-up
- Rate of discontinuation of control treatment (statin) 0%
- Control event rate: 0.065
- Hazard ratio: 0.75
- Delayed treatment effect: begins at 3 months after randomization, full effect at 6 months after randomization

Estimated Power	Rate of discontinuation of blinded therapy (active)	Number of Primary Events	Estimated Trial Duration from First Patient Randomized
85%	0.080	841	80 months
	0.085	867	82 months
	0.090	904	85 months

5.7 Sample Size Conclusions:

The original design with a sample size of 3,300 results in a well-powered trial for detecting the specified difference in the original primary endpoint proposed for AIM-HIGH (E4). This will result in 99% power to detect a difference between hazard rates of 0.067 and 0.089 (hazard ratio=0.75) in the simvastin+niacin and simvastatin therapy groups, respectively.

Moreover, there is 68% power to detect a difference in the secondary AIM-HIGH efficacy endpoint (E3) with this sample size.

The trial, as modified, is adequately powered to detect a 25% relative risk reduction (0.75 hazard ratio) in the revised primary outcome, E5, as described above. Patients will be followed to a common termination date approximately 80 months from the first patient randomized until 850 primary outcome events have been observed. These assumptions

depend on the rate of discontinuation of blinded therapy remaining at or less than 0.08. If overall rates of discontinuation of blinded therapy higher than this are observed, the trial may be extended in order to maintain adequate power.

6. Regulatory Standards

6.1 Informed consent

The investigator, or a person designated by the investigator, should fully inform the patient of all pertinent aspects of the clinical trial including the written information approved by the Ethics Committee.
Prior to a patient's participation in the clinical trial, the Informed Consent Form should be signed and personally dated by the patient or by the patient's legally acceptable representative.

The Informed Consent Form must be reviewed and approved by the sponsor prior to submission to the appropriate Ethics Committee for approval.

6.2 Institutional Review Board/Independent Ethics Committee (IRB/IEC)

The investigator must submit this protocol to the appropriate IRB/IEC, and is required to forward to the Sponsor a copy of the written and dated approval opinion by the Chairman with IRB/IEC composition.

The study (study number, protocol title and version number), the document reviewed (protocol, Informed Consent Form, Investigator's Brochure, etc.) and the date of the review should be clearly stated on the written IRB/IEC approval opinion.

During the clinical trial, any amendment or modification to the protocol should be sent to the IRB/IEC. It should also be informed of any event likely to affect the safety of patients or the continued conduct of the study, in particular any change in safety and all updates to the Investigator's Brochure will be sent to IRB/IEC.

7. Study Monitoring

7.1 Responsibilities of the Investigator(s)

The investigator(s) undertake(s) full responsibility to perform the study in accordance with this protocol, Good Clinical Practice and the applicable regulatory requirements.

The investigator is required to ensure adherence with the visit schedule and procedures required by the protocol. The investigator agrees to provide all information requested in the Case Report Form in an accurate and legible manner.

7.2 Responsibilities of the Clinical Trial Coordinating Center

The Clinical Trial Coordinating Center (CTC) for the study is responsible to Health Authorities for taking all reasonable steps to ensure the proper conduct of the study as regards ethics, protocol compliance, integrity and validity of the data recorded on the Case Report Forms, in keeping with established Good Clinical Practice (GCP) standards. Therefore, the main duty of the Monitoring Team is to help the investigator and the CTC maintain a high level of ethical, scientific, technical and regulatory quality in all aspects of the study.

At regular intervals during the study, the clinical centers will be contacted, through site visits, letters, or telephone calls, by a representative of the Monitoring Team to review study progress, investigator and patient compliance to protocol requirements and any emergent problems. During monitoring visits, the following points will be scrutinized with the investigator: patient informed consent, patient recruitment and follow-up, study drug allocation, patient compliance with the investigational medicine, investigational medicine accountability, concomitant therapy use, adverse event documentation and reporting, and quality of data.

8. Summary

Advances in dyslipidemic therapy, which have contributed to the decline in incident CHD over the past 30 years, have been largely attributable to the statins. However, despite these impressive gains, CHD remains the most frequent cause of death in the United States and the Western World. Although large-scale clinical trials with statins have found that reducing LDL-C decreases mortality and coronary events by 25 – 35%, event rates in these trials remain unacceptably high, in the range of 70% to 75% of those observed among placebo-treated patients. Furthermore, nearly 30 years ago, results from the Coronary Drug Project showed the benefits of niacin treatment in decreasing cardiovascular events, also by 25 – 35%, in patients with previous myocardial infarction. This benefit has been attributed to, among other effects of niacin, changes in HDL-C and triglycerides. More recently, the accumulated clinical evidence has led many to suggest that low HDL-C should be considered a target for therapy, particularly in patients with multiple risk factors, established CHD, or its equivalent. Therefore, now is the time to capitalize on the potential to achieve an additive event rate reduction through combination therapy and test this hypothesis in a long term, large scale clinical outcomes trial.

Raising HDL-C levels has been shown to reduce coronary events in CHD patients at or near LDL-C goals. Niacin controls multiple lipid and lipoprotein abnormalities and is presently the most effective agent for raising low levels of HDL-C. Combination therapy using niacin and a statin can provide complementary benefits to the serum lipid profile. Since patients with vascular disease (CHD, cerebrovascular disease, or PAD) who have so-called mixed dyslipidemia are at very high risk for developing subsequent MI, stroke or ischemic limb loss, The AIM-HIGH Trial may shed important light on defining

optimal lipid management for these patients. Such multidimensional dyslipidemic therapy may provide clinicians with a powerful approach to treating patients whose risk for developing CHD, cerebrovascular disease, or PAD may not be mitigated by lowering LDL-C alone, especially in those individuals who have residual low levels of HDL-C and/or elevated levels of triglycerides.

As noted previously, no randomized clinical trial to date has addressed systematically the dyslipidemic management of patients with symptomatic vascular disease, including patients with CHD, cerebrovascular disease, and PAD as expressions of diffuse systemic atherothrombosis. The inclusion of patients with diabetes, metabolic syndrome and the atherogenic dyslipidemic triad of low HDL-C, elevated TG and increased small, dense LDL particles, many of whom are obese and at significant risk for subsequent vascular complications, provides a compelling rationale for configuring a therapeutic strategy aimed at reducing the high-risk associated with these overlapping atherothrombotic conditions.

If the AIM-HIGH Trial can prove the hypothesis that combination dyslipidemic therapy directed toward multiple lipid targets improves significantly cardiovascular and cerebrovascular events compared to statin monotherapy, it will provide a scientifically-important and clinically-meaningful approach to optimizing event-free survival in a large and growing population of high risk patients for whom treatment, at present, is less than adequate.

In summary, both the healthcare consequences (morbidity and mortality) as well as the economic consequences (rising healthcare expenditures and spiraling direct/indirect costs) of this therapeutic challenge in dyslipidemic management have profound healthcare delivery—and potentially healthcare policy—implications for government organizations in the U.S., Canada, and worldwide.

9.0 References

1. American Heart Association. 2002 Heart and Stroke Statistical Update. Dallas, Texas: 2002
2. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation*. 2002;106:3143-421.
3. Kwiterovich PO, Jr. The metabolic pathways of high-density lipoprotein, low-density lipoprotein, and triglycerides: a current review. *Am J Cardiol*. 2000;86:5L-10L.
4. Lamarche B, Tchernof A, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, Despres JP. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. *Circulation*. 1997;95:69-75.
5. Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation*. 1990;82:495-506.
6. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes*. 1988;37:1595-607.
7. Brunzell JD. Dyslipidemia of the metabolic syndrome. In: Eckel R, ed. *Obesity: Mechanisms and Clinical Management.* New York: Lippincott Williams & Wilkins; 2002:378-398.
8. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA* 2002;287:356-9.
9. Austin MA, McKnight B, Edwards KL, Bradley CM, McNeely MJ, Psaty BM, Brunzell JD, Motulsky AG. Cardiovascular disease mortality in familial forms of hypertriglyceridemia: A 20-year prospective study. *Circulation*. 2000;101:2777-82.
10. Miettinen TA, Gylling H. Cholesterol absorption efficiency and sterol metabolism in obesity. *Atherosclerosis*. 2000;153:241-8.
11. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med*. 1998;339:229-34.
12. McNeely MJ, Edwards KL, Marcovina SM, Brunzell JD, Motulsky AG, Austin MA. Lipoprotein and apolipoprotein abnormalities in familial combined hyperlipidemia: a 20-year prospective study. *Atherosclerosis*. 2001;159:471-81.
13. Taskinen MR. Quantitative and qualitative lipoprotein abnormalities in diabetes mellitus. *Diabetes*. 1992;41 Suppl 2:12-7.
14. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20536 high-risk individuals: a randomised placebo-controlled trial. *Lancet* 2002;360:7-22.
15. Cannon CP, Braunwald E, McCabe CH, et al. Comparison of intensive and moderate lipid lowering with statins after acute coronary syndromes. *N Engl J Med* 2004;350:15.

16. Rubins HB, Robins SJ, Collins D, Iranmanesh A, Wilt TJ, Mann D, Mayo-Smith M, Faas FH, Elam MB, Rutan GH, et al. Distribution of lipids in 8,500 men with coronary artery disease. Department of Veterans Affairs HDL Intervention Trial Study Group. *Am J Cardiol*. 1995;75:1196-201.
17. Castelli WP. Cholesterol and lipids in the risk of coronary artery disease--the Framingham Heart Study. *Can J Cardiol*. 1988;4 Suppl A:5A-10A.
18. Rubins HB, Robins SJ, Collins D, et al. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. N Engl J Med 1999;341:410-8.
19. Brown BG, Zhao XQ, Chait A, Fisher LD, Cheung MC, Morse JS, Dowdy AA, Marino EK, Bolson EL, Alaupovic P, Frohlich J, Albers JJ. Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N Engl J Med*. 2001;345:1583-92.
20. Capuzzi DM, Morgan JM, Brusco OA, Intenzo CM. Niacin dosing: relationship to benefits and adverse effects. *Curr Atheroscler Rep* 2000;2:64-71.
21. Knopp RH. Evaluating niacin in its various forms. *Am J Cardiol*. 2000;86:51L-56L.
22. Boden WE. Therapeutic implications of recent ATP III guidelines and the important role of combination therapy in total dyslipidemia management. *Curr Opin Cardiol*. 2003;18:278-85.
23. Kashyap ML, McGovern ME, Berra K, Guyton JR, Kwiterovich PO, Harper WL, Toth PD, Favrot LK, Kerzner B, Nash SD, Bays HE, Simmons PD. Long-term safety and efficacy of a once-daily niacin/lovastatin formulation for patients with dyslipidemia. *Am J Cardiol*. 2002;89:672-8.
24. Van JT, Pan J, Wasty T, Chan E, Wu X, Charles MA. Comparison of extended-release niacin and atorvastatin monotherapies and combination treatment of the atherogenic lipid profile in diabetes mellitus. *Am J Cardiol*. 2002;89:1306-8.
25. Jin F-Y, Kamanna VS, Kashyap ML. Niacin decreases removal of high-density lipoprotein apolipoprotein A-I but not cholesterol ester by Hep G2 cells: implication for reverse cholesterol transport. *Arterioscler Thromb Vasc Biol* 1997;17:2020-8.
26. Kamanna VS, Kashyap ML. Mechanism of action of niacin on lipoprotein metabolism. *Curr Atheroscler Rep* 2000;2:36-46.
27. Jacobson TA, Chin MM, Fromell GJ, Jokubaitis LA, Amorosa LF. Fluvastatin with and without niacin for hypercholesterolemia. *Am J Cardiol*. 1994;74:149-54.
28. Stein EA, Davidson MH, Dujovne CA, Hunninghake DB, Goldberg RB, Illingworth DR, Knopp RH, Miller VT, Frost P, Isaacsohn JL, Mitchel YB, Melino MR, Shapiro D, Tobert JA. Efficacy and Tolerability of Low-dose Simvastatin and Niacin, Alone and in Combination, in Patients With Combined Hyperlipidemia: A Prospective Trial. *J Cardiovasc Pharmacol Ther*. 1996;1:107-116.
29. Wolfe ML, Vartanian SF, Ross JL, Bansavich LL, Mohler ER, 3rd, Meagher E, Friedrich CA, Rader DJ. Safety and effectiveness of Niaspan when added sequentially to a statin for treatment of dyslipidemia. *Am J Cardiol*. 2001;87:476-9, A7.

30. Capuzzi DM, Morgan JM, Weiss RJ, Chitra RR, Hutchinson HG, Cressman MD. Beneficial effects of rosuvastatin alone and in combination with extended-release niacin in patients with a combined hyperlipidemia and low high-density lipoprotein cholesterol levels. *Am J Cardiol*. 2003;91:1304-10.
31. Gibbons LW, Gonzalez V, Gordon N, Grundy S. The prevalence of side effects with regular and sustained-release nicotinic acid. *Am J Med* 1995;99:378-85.
32. Knopp RH. Drug treatment of lipid disorders. *N Engl J Med* 1999;341:498-511.
33. Capuzzi DM, Morgan JM, Brusco OA, Jr., Intenzo CM. Niacin dosing: relationship to benefits and adverse effects. *Curr Atheroscler Rep* 2000;2:64-71.
34. Dalton TA, Berry RS. Hepatotoxicity associated with sustained-release niacin. *Am J Med* 1992;93:102-4.
35. Patterson DJ, Dew EW, Gyorkey F, Graham DY. Niacin hepatitis. *South Med J* 1983;76:239-41.
36. Rader JI, Calvert RJ, Hathcock JN. Hepatic toxicity of unmodified and time-release preparations of niacin. *Am J Med* 1992;92:77-81.
37. Clementz GL, Holmes AW. Nicotinic acid-induced fulminant hepatic failure. *J Clin Gastroenterol* 1987;9:582-4.
38. Knopp RH. Clinical profiles of plain versus sustained-release niacin (Niaspan) and the physiologic rationale for nighttime dosing. *Am J Cardiol* 1998;82:24U-8U.
39. Knopp RH, Alagona P, Davidson M, et al. Equivalent efficacy of a time-release form of niacin (Niaspan) given once-a-night versus plain niacin in the management of hyperlipidemia. *Metabolism* 1998;47:1097-104.
40. Guyton JR, Goldberg AC, Kreisberg RA, Sprecher DL, Superko HR, O'Connor CM. Effectiveness of once-nightly dosing of extended-release niacin alone and in combination for hypercholesterolemia. *Am J Cardiol* 1998;82:737-43.
41. American Diabetes Association. Management of dyslipidemia in adults with diabetes. *Diabetes Care* 2002;25(suppl 1):S74-S77.
42. Garg A, Grundy SM. Nicotinic acid as therapy for dyslipidemia in non–insulin-dependent diabetes mellitus. *JAMA* 1990;264:723-6.
43. Elam MB, Hunninghake DB, Davis KB, et al. Effect of niacin on lipid and lipoprotein levels and glycemic control in patients with diabetes and peripheral arterial disease. The ADMIT study: a randomized trial. *JAMA* 2000;284:1263-70.
44. Grundy SM, Vega GL, McGovern ME, et al. Efficacy, safety, and tolerability of once-daily niacin for the treatment of dyslipidemia associated with type 2 diabetes: results of the assessment of diabetes control and evaluation of the efficacy of Niaspan trial. *Arch Intern Med* 2002;162:1568-76.
45. Morse JS, Brown BG, Zhao X-Q, et al. Niacin plus simvastatin protect against atherosclerosis progression and clinical events in CAD patients with low HDLc and diabetes mellitus or impaired fasting glucose [abstract 842-3]. *J Am Coll Cardiol* 2001;37(suppl A):262A.
46. Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial Infarction redefined--a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *J Am Coll Cardiol.* 2000;36(3):959-969.
47. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. *Biometrics.* 1979;35:549-556.

48. Lan KKG, DeMets DL. Discrete sequential boundaries for clinical trials. *Biometrika.* 1983;70:659-663.
49. Lachin JM, Foulkes MA. Evaluation of Sample Size and Power for Analyses of Survival with Allowance for Nonuniform Patient Entry, Losses to Follow-up, Noncompliance and Stratification. *Biometrics.* 1986;42:507-519.
50. Scandinavian Simvastatin Survival Study Group. Randomised Trial of Cholesterol Lowering in 4,444 Patients with Coronary Heart Disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet.* 1994;344:1383-1380
51. Lakatos E. Sample sizes based on the log-rank statistic in complex clinical trials. Biometrics 44:229-241, 1988.

APPENDICES

Appendix 1. Intima-Media Thickness Measurement

Carotid intima-media thickness (IMT) is a marker of early arterial change of the arterial walls including atherosclerosis and/or vascular hypertrophy, detected by B-Mode ultrasonography.

1. Recommendations for the ultrasonographic examinations:

- Machines equipped with 5 or 7 MHz transducers
- Subjects in the supine position
- ECG signal used for synchronizing the image analysis to the end of the diastole
- Doppler ultrasound used for vessel identification (and information on blood flow velocity)
- Carotid artery scanned at the level of the bifurcation, with the head turned to the opposite side (e.g. to the right for left carotid artery)
- Examined region:
    - 30 mm of the common carotid artery
    - carotid bulb
    - 10 mm each of the internal and external carotid arteries
- Regions scanned with both longitudinal and transverse projections, in order to assess the occurrence of plaques
- Three " frozen" images recorded for assessment of intima-media thickness and lumen diameter
- Optimal image projection considered to be achieved when ultrasound beams are perpendicular to the far vessel wall

2. Recommendations for assessment of intima-media thickness (defined as the distance from the leading edge of the lumen—intima interface to the leading edge of the media-adventitia interface of the arterial wall) and lumen diameter (defined as the maximal distance between the leading edges of the intima-lumen interface)

- Ultrasonographic images analyzed with a computerized system
- Intima-media thickness measured in a 10-mm long segment just proximal to the carotid bulb in the common carotid artery
- Calculation by the computer program of the minimum, maximum and mean values of intima-media thickness from three separate images

3. Assessments of plaques

- **A plaque is defined as a distinct area with an intima-media thickness exceeding twice that of the neighboring sites**
- Classification of plaques, according to a four-graded semi-quantitative scale of their size/severity:
    - Grade 0: no plaque
    - Grade 1: small localized plaque/wall thickening

    - Grade 2: moderate plaque with <50% lumen diameter stenosis
    - Grade 3: circumferential and/or large plaque with ≥ 50% lumen diameter stenosis
- Plaques detection must be focused on the distal part of the common carotid artery, the carotid bulb or in the proximal parts of the internal or external carotid artery.

Appendix 2. Criteria for Carotid Stenosis ≥ 70%

1. Ultrasound

- Technique
  - Color duplex ultrasound scanners equipped with 5 or 7 MHz linear-array probe
  - Examination in transverse and longitudinal section using B-mode grey-scale imaging, then with color Doppler ultrasound, of the following:
    - Common carotid artery (CCA)
    - Carotid bifurcation
    - Extracranial internal carotid artery (ICA)

- Doppler waveforms obtained from
  - The base of the CCA
  - The Bulb of the ICA
  - The distal end of the extracranial ICA
  - And at the sites of suspected significant stenosis

- Criteria used for classifying the degree of stenosis

Category	Diagnostic Criteria
Normal	No visible plaque and normal waveforms
Stenosis	
1-29%	Visible plaque causing < 30% diameter stenosis and/or spectral broadening/flow disturbance on waveform
30-49%	Visible plaque causing 30-49% diameter stenosis, peak systolic velocity (PSV) < 1.2 m/s
50-69%	PSV ≥ 1.2m/s
70-99%	PSV ≥ 3m/s and end-diastolic velocity > 1.2m/s or very narrow lumen with damped flow distally
Total Occlusion	No flow detected

2. Intra-arterial digital subtraction angiography (IADSA)

- Technique
  - 5F catheter introduced via the femoral artery under local anesthesia
  - Selective catheterization of the CCA (contrast medium injected in the innominate artery or the aortic arch, in case of proximal vessel occlusion)
  - IADSA images acquired at 2/s
  - Two views of the carotid bifurcation (anteroposterior oblique and lateral)
  - Three views of the intracranial circulation (Towne's, Towne's oblique and lateral)

- Measurement of degree of stenosis
    - Degree of ICA stenosis measured on magnified hard copy films of either the oblique or the lateral image, whichever shows the most severe stenosis
    - Diameter of the residual lumen compared with the original diameter of the normal carotid bulb extrapolated on the angiogram
    - Percentage stenosis = (normal lumen-residual lumen)/normal lumen X 100
    - Grading systems:
        - Normal
        - Mild (1-29%)
        - Moderate (30-69%)
        - Severe (70-99%)
        - Total occlusion

Appendix 3. Ankle-Brachial Index Measurement

- Measure highest systolic reading in both arms
    - Record first droppler sound as cuff is deflated
    - Record at the radial pulse
    - Use highest of the two arm pressures

- Measure systolic readings in both legs
    - Cuff applied to calf
    - Record first Doppler sound as cuff is deflated
    - Use Doppler untrasound device
        - Record dorsalis pedis pressure
        - Record posterior tibial pressure
    - Use highest ankle pressure (DP or PT) for each leg

- Calculate ratio of each ankle to brachial pressure
    - Divide each ankle by highest brachial pressure

Schedule of Laboratory Assessments

Appendix 4

Appendix 4. Schedule of Laboratory Assessments
(See Laboratory Manual of Operations for Additional Detail)

															Clinically Suspected Toxicity					
															Myopathy			Hepatic		
	Screen	Base-line	1 mo	3 mo	6 mo	9 mo	1Yr	18 mo	2 yr	30 mo	3 yr	42†† mo	4‡ yr	Final (note 15)	Init.¶ Samp	1st FU	2nd FU	Int.¶ Samp	1st FU	2nd FU
DBQ (1)	all		all	all	all		all		all		all		all	all	-	-	-	-	-	-
HDL2,3	all		-	-	-	-	all				all		all	-	-	-	-	-	-	-
ApoB (2)		all	-	-	-	-	all				all			-	-	-	-	-	-	-
ApoA-I		all	-	-	-	-	all				all			-	-	-	-	-	-	-
ApoCIII (3)		15%	-	-	-	-	15%				15%			-	-	-	-	-	-	-
ApoCIIIhp		15%	-	-	-	-	15%				15%			-	-	-	-	-	-	-
Lipoparticles(11)		all	-	-	-	-	all							-	-	-	-	-	-	-
Lp(a)		all	-	-	-	-	all				-			-	-	-	-	-	-	-
TSH		all	-	-	-	-	-				-			-	-	-	-	-	-	-
CK (4)	all		-	5%*	-	-	5%				-			-	5%*	5%*	5%	3%*	-	-
Fibrinogen		15%	-	-	-	-	15%				-									
Uric Acid (5)	all		-	5%	-	-	5%				-			-	-	-	-	-	-	-
Insulin (6)		all	-	30%	-	-	all				all			-	-	-	-	-	-	-
HGB A1c (7)		all	-	30%	-	-	45%				45%			-	-	-	-	3%*	-	-
Glucose (8)	all		30%	30%	-	-	all		all		all		all	all	-	-	-	-	-	-
Homocyst (9)		all	-	10%	-	-	10%				10%			-	-	-	-	-	-	-
AST (10)	all		20%	all	all	-	all	all	all	all	all	all	all	all	3%*	-	-	3%*	3%*	3%*
hsCRP (12)		15%	-	-	-	-	all				all			-	-	-	-	-	-	-
Creatinine (13)	all		-	all	-	-	all				all			-	-	-	-	-	-	-
Retained samples (14)		all					all				all									
HDL proteomics/MRI substudies†		all					all		all											

Table footnotes:

+ Final sample is that obtained at close-out visit just before therapy is discontinued).

*** All of those with baseline elevation > lab ULN or with suspect symptoms on-therapy (assume 3% of 3300 population).**

¶ An initial sample of CK and AST will be drawn at the first presentation with symptoms consistent with hepatic or myotoxicity and, if abnormal, will be repeated at least twice with frequency to be determined by the judgment of the investigator and the patient's primary physician.

††And at each mid-year visit thereafter until study end ‡And at the annual study visit thereafter until study end

(1) – Derived beta quant is measured TC, TG, HDL-C by precipitation, and LDL computed by Friedewald.
(2) – ApoB and ApoAI to be measured in all patients at baseline, 1 year, and 3 years.
(3) – ApoCIII total and that in the heparin-precipitate (hp) fraction (apoB-associated) in estimated 15% of patients not on statins at baseline.
(4) -- CK measured at baseline in all, at 1 month on-therapy and at 1 yr in asymptomatic subgroup with CK >2x ULN at baseline (assume 5% of 3300), and in all with new onset muscle aches (assume another 5%), and also, initially, with suspected hepatotoxicity.
(5) – Uric acid measured at baseline and in an assumed 5% with initial levels >7.5 mg/dL (445 umol/L) at 1 year or any with new gout symptoms.
(6–8) - Insulin, HbA1C, and glucose measured in all at baseline, and (in an estimated 30% with abnormal HgbA1C levels at baseline) early post-randomization. Insulin will be remeasured in all patients at years 1 and 3. Fasting glucose will be obtained in all patients annually and at the final study visit. HbA1C will be obtained at years 1 and 3 in an estimated 30% who have abnormal levels at baseline plus a representative 15% sample from patients whose baseline HbA1C is normal (i.e., in an estimated total of 45% of patients at years 1 and 3)..
(9) – Homocysteine at baseline in all, and in an estimated 10% of those with baseline >15 mg/dL (110 umol/L) at 1 and 3 yr.
(10) – AST in all at baseline, 3 months and every 6 months thereafter, and within 3 months after starting combination of simvastatin + ezetimibe in conjunction with blinded therapy or after starting 80 mg simvastatin, and also in initial sample for all with suspected myopathy and all samples with suspected hepatic toxicity.
(11) – Lipoprotein particle size/concentration (by NMR) in all patients at baseline and at year 1.
(12) – hsCRP should be measured by the Dade-Behring reagent, or its equivalent. Measurement will be made in estimated 15% of patients not on a statin at baseline, and in everyone at years 1 and 3.
(13) – Creatinine will be measured at baseline, and at 1 and 3 yrs
(14) – Samples to be frozen and stored. See laboratory Manual of Operations for additional detail.
(15) Final visit labs will be collected at end of double-blind treatment period and at end of extension to follow-up, approximately 18 months after end of double-blind treatment.

†For patients and sites participating in the MRI and HDL proteomics substudies (see Appendices 5 and 6) after August 1, 2009. Blood for HDL proteomics substudy at baseline and years 1 and 2. Serum creatinine at baseline, years 1 and 2 prior to MRI scans.

MRI Substudy

Appendix 5

AIM-HIGH Carotid MRI Study Protocol Summary

I. Overall Objectives

The overall objectives of this study are to examine the effect of intensive LDL-lowering plus HDL-raising therapy, compared with LDL-lowering alone, on atherosclerotic plaque lipid composition and burden in carotid arteries assessed by multi-contrast MRI and to determine if MRI based plaque characterization including tissue composition and volume predict future clinical cardiovascular events. **The primary MRI endpoint is the mean change in plaque lipid composition over 2 years.** The secondary endpoints include plaque volume and a series of plaque characteristic indexes of wall thickness, tissue components including calcium, loose matrix, hemorrhage and fibrous tissue.

II. Specific Aims

(1) To test the primary hypothesis that compared with LDL-lowering alone, intensive LDL-lowering plus HDL-raising therapy decreases the mean plaque lipid composition in carotid arteries assessed by MRI, and the lipid-rich plaque identified by MRI is more likely to have a volume change in response to therapy. To achieve this goal, we will (a) perform carotid MRI scans in 300 study subjects enrolled in the *A*therothrombosis *I*ntervention in *M*etabolic Syndrome with Low *H*DL/High Triglycerides and *I*mpact on *G*lobal *H*ealth Outcomes (AIM-HIGH) trial at baseline, 1 year, and 2 years post randomization; (b) perform quantitative assessments of carotid plaque lipid composition blinded to the MRI time sequence and treatment; (c) examine the association between plaque lipid composition at baseline and volume change over 2 years; (d) compare the change in plaque lipid composition over 2 years between LDL-lowering alone and LDL-lowering plus HDL-raising groups.

(2) To test the hypothesis that compared with LDL-lowering alone, intensive LDL-lowering plus HDL-raising therapy decreases plaque burden, volume and wall thickness. To achieve this goal, we will (a) perform quantitative assessments of plaque volume and carotid vessel wall thickness blinded to the MRI time sequence and treatment; (b) compare the mean change in carotid plaque volume and wall thickness over 2 years between LDL-lowering alone and LDL-lowering plus HDL-raising groups.

(3) To test the hypothesis that increased plaque lipid composition or vessel wall thickness by MRI is associated with increased risk of cardiovascular events. To achieve this goal, we will (a) utilize the data on cardiovascular events which include CHD death, fatal/non-fatal MI or stroke, and hospitalization/revascularization for acute coronary syndrome that will be collected in the AIM-HIGH main trial; (b) determine if increased carotid plaque lipid composition or vessel wall thickness at baseline or its change during 2 years of therapy are statistically associated with cardiovascular events; (c) identify meaningful plaque characteristics and their change by examining the statistical association between each of the plaque characteristics (volume, wall thickness, lipid content and other tissue contents) at baseline and two years, and their change during therapy and occurrence of cardiovascular events.

(4) To examine the association of factors which include clinical risk factors, lipids, lipoprotein heterogeneity, inflammatory markers and carotid plaque characteristics. To achieve this goal, we will (a) utilize laboratory data on lipids, lipoprotein heterogeneity, and inflammatory markers both at baseline and on therapy collected in the AIM-HIGH main trial; (b) describe the change in each of these measurements between baseline

and on treatment among the 220 patients in the proposed MRI sub-study; (c) determine the statistical significance of each measured risk variable and its change in relation to the change in plaque characteristics.

III. Carotid MRI 3T Protocol

The carotid MR scans for this study will be performed on a GE 3T or a Philips 3T whole body scanner located at [scanner location], one of the AIM-HIGH study designated MRI centers. Bilateral carotid artery MR scans will be obtained at baseline, 1 year and 2 years.

Patients will be placed in the supine position in the MR scanner with the neck extended to bring the carotid arteries into a more superficial location relative to the skin. A custom designed head holder is used to minimize patient movement. Two separate phased-array carotid coils are used for simultaneous bilateral carotid imaging. A standard 3-plane localizer is used to identify the carotid arteries. A 2-D TOF sequence is applied as a localizer to identify both the right and left common carotid bifurcation (flow divider) and to obtain high quality blood flow and vessel wall imaging. Two 2-D fast spin echo (FSE) scans are acquired, one with PD and the other with T2. Following the FSE scan, a 2-D spin echo (SE) technique is applied to acquire a set of cross-sectional images with T1. The longitudinal coverage of this set of images is centered at the carotid bifurcation and covers the entire most likely diseased region. These scans have exactly the same spatial coverage as the SE scans with identical image locations. One-half dose (0.05 mmoles per kilogram body weight) of gadolinium contrast material, Magnevist, will be administrated intravenously though a power injector. Images of 4 locations, centered either on the carotid bifurcation or on the plaque, will be simultaneously acquired using axial 2D spoiled gradient-recalled echo imaging without cardiac gating. These images are obtained at 10 time points separated by a repetition interval of 15sec. Post-contrast T1-weighted images will be acquired 5-7 minutes after gadolinium contrast administration. The total scan time for each patient is about 50 minutes. The imaging parameters used in these scans are summarized in Table below.

Table. MR Imaging Parameters

MRI Sequence	**3-plane**	**T of F**	**D-IR**	**PDW**	**T2W**	**T1W**	**T of F**	**SPGR**	**T1W**
Scan plane		Axial	Oblique	Axial	Axial	Axial	Axial	Axial	Axial
Contrast agent	-	-	-	-	-	-	-	+	+
Image mode		2-D	2-D	2-D	2-D	2-D	3-D	2-D	2-D
TR (ms)		MIN	1800	4000	4000	800	20-23	80	800
TE (ms)		MIN	MIN	MIN	50	MIN	MIN	MIN	MIN
Field of view (cm)		14-16	16	14-16	14-16	14-16	14-16	14-16	14-16
Matrix size		256x128	256x256	384x384	384x384	384x384	384x384	256x256	384x384
Slice thickness (mm)		2	2	2	2	2	2	2	2
Features			***	***	***	***	***	***	***

***: Fat saturation and flow suppression.

Renal function monitoring: A gadolinium agent used in this study is FDA-approved. However, in a very small percentage of patients with impaired kidney function, gadolinium has been suspected to cause a new disease, called nephrogenic systemic fibrosis, or nephrogenic fibrosing dermopathy (NSF/NFD). To monitor the renal function, calculated creatinine clearance, glomerular filtration rate (GFR), will be measured within 4 weeks of use of gadolinium agent during the carotid MRI scans. If

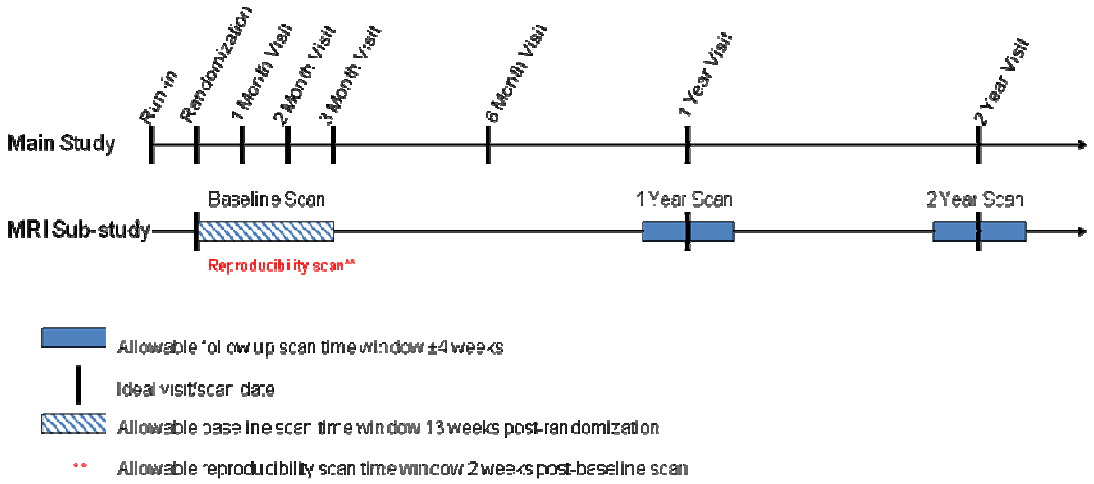
GFR<60ml/min/1.73m^2 is found at any measurements, or a ≥50% drop in GFR from the previous testing point, the subject will be excluded from the study and renal function measurement will be repeated and recorded, if necessary, the subject will be referred to nephrology for further evaluation and treatment.

IV. MRI Reproducibility Study

Five subjects at each of the participating MRI centers will receive two identical carotid MRI scans within 2 weeks. A total of 80 subjects will be enrolled into this study. This will help us to (a) determine the impact of site on reproducibility of plaque burden and compositional measurements and (b) determine the impact of platform (GE and Philips 3T whole body scanners) on reproducibility of the plaque measurements.

All subjects willing to participate in this reproducibility study will be rechecked on their renal function before the repeat scan. The same renal function criteria and monitoring plan in section III will be applied to this reproducibility study.

V. Carotid MRI Scan-timeline in AIM-HIGH



VI. MRI Analysis Protocol

1. Image Blinding and Matching Process

The original MRI examination identification (ID) number, image date/time, and series information will be replaced with a new and randomly generated MRI analysis ID number. A master log file containing both original and new image information, and study subject ID will be generated at the time of renaming. This blinding process will ensure that MRI reviewers are fully blinded to patient information and time sequence during the image analysis. It will also protect patient confidentiality in accordance to HIPPA guidelines.

Prior to image review, all across-sectional images from TOF, T1, PD, T2, and post-CE T1 weightings will be co-registered using the carotid bifurcation as a physical landmark.

2. Visual Assessment of Atherosclerotic Lesion Type

A previously published MRI-based AHA lesion classification scheme will be used for this evaluation: type I-II = near-normal wall thickness; type III = diffuse wall thickening or small eccentric plaque; type IV-V = plaque with a necrotic core; type VI = complex plaque with a possible surface defect, hemorrhage, or thrombus; type VII = calcified plaque; and type VIII = fibrotic plaque without a necrotic core.

3. Quantitative Measurements of Plaque Volume and Tissue Composition

(a) Plaque Volume Measurement: Contours will be placed around the lumen and outer-wall boundaries of carotid artery. These contours can be created manually or automatically. The arterial wall area = outer-wall area – lumen area. The wall volume is calculated as: wall area X 2 mm (slice thickness). Wall/outer wall ratio will be used as a

normalized wall index that is adjusted for differences in carotid artery size in the common carotid, bifurcation, and internal carotid arteries.

(b) Plaque Tissue Content Measurement Using Automated Plaque Tissue Segmentation: As introduced in the preliminary studies, the Vascular Imaging Laboratory at the University of Washington has developed an semi-automated system (MEPPS) and has shown that MEPPS is capable of achieving accuracy similar to results achieved by manual review by expert reviewers for quantifying plaque composition. The automated segmentation is based on the fact that various tissue contents such as lipid, calcium, loose matrix and fibrous tissue have different signal characteristics from each weighting as shown in the Table below. The system first determines the probability that each MRI pixel belongs to each of the 4 tissue types (lipid, calcification, loose matrix, and fibrous tissue). Then, it uses the competing active contours to identify the boundaries of high-probability regions for each tissue type.

Table. Tissue Classification Criteria

	TOF	T1W	PDW	T2W	>80% SI↑Post contrast T1W
LRNC with (A) No or little Hemorrhage	o	o/+	-/o	-/o	-
(B) Fresh Hemorrhage	+	+	-/o	-/o	-
(C) Recent Hemorrhage	+	+	+	+	+
Calcification	-	-	-	-	-
Loose Matrix	o	-/ o	+	+	+
Dense (Fibrous) Tissue	-	o	o	o	+

The classification into the subgroups is based on the following signal intensities (SI) relative to adjacent muscle. LRNC = Lipid-Rich/Necrotic Core. + = hyper-intense, o = iso-intense, - = hypo-intense.

The steps for measuring plaque volume and automated segmentation include followings:

Step 1. Image matching: After loading the 5 MRI sequences (pre-contrast T1, post-contrast T1, T2, PD, TOF) onto the CASCADE computer interface, the images are manually matched to each other by using the carotid bifurcation as a landmark. An example is shown in Figure 1 below.

Step 2. Lumen and wall boundary detection: Using information available from the multi-contrast weighted images, the lumen and wall boundary of the carotid artery are identified and outlined by expert reviewers. An example is shown in Figure 2 with lumen boundary in red and outer wall boundary in blue.

Step 3. Registration: After manual boundary detection, the CASCADE Program will automatically identify and outline the lumen and wall boundary of the carotid artery in the remaining 4 sequences. An example is shown in Figure 3 below. Expert readers will review the outlines generated by CASCADE, and have the opportunity to manually override errors in the automated outlines which may occur as a result of marginal image quality or flow artifact.

Step 4. Auto-segmentation: The CASCADE Program will automatically identify and quantify the tissue component within the arterial wall. Expert readers will then review the output of CASCADE to insure correct classification of the plaque

components. An example is shown in Figure 4 below with loose matrix in purple and lipid content in yellow.

The image analysis for this study will be performed at the time of completing both MRI examinations. By doing so, a region of each carotid artery, covered by both baseline and 2-year scans, will be identified and image analysis will be performed within the region. This will allow the same reviewers to analyze all images and therefore to reduce the inter-reader variability. The analysis will be performed at Vascular Imaging Lab using CASCADE with automated tissue segmentation capability using MEPPS.

(c) Plaque Tissue Composition: This will be calculated based on each identified tissue volume and wall volume at each given location: tissue volume/wall volume X (100%), and presented as percentage.

(d) Summary of Plaque Variables: CASCADE can produce a list of comprehensive plaque assessments. Plaque burden measurements will include the carotid lumen, wall, and outer-wall area in mm2, and wall thickness in mm, and wall/outer-wall ratio (a normalized wall index that is adjusted for carotid artery size difference) that is equivalent to the % of atheroma area used in the coronary intravascular studies. The plaque tissue characteristics will be presented as absolute measurement in mm2 and as a proportion of the corresponding vessel wall area, expressed as in % lipid, % loose matrix, % calcium, % fibrous tissue

Figure 1.

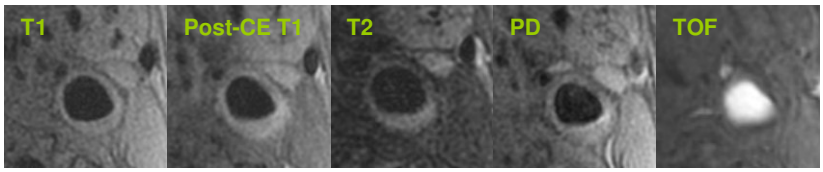


Figure 2.

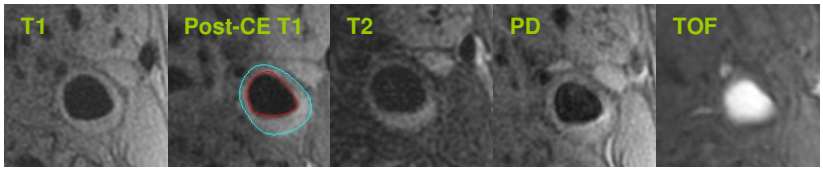


Figure 3.

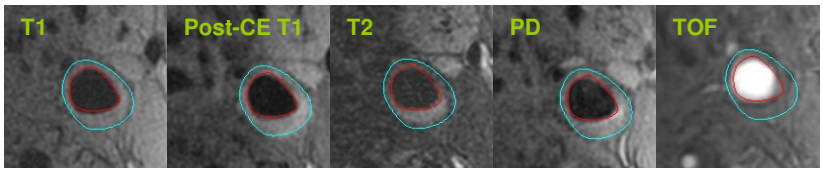
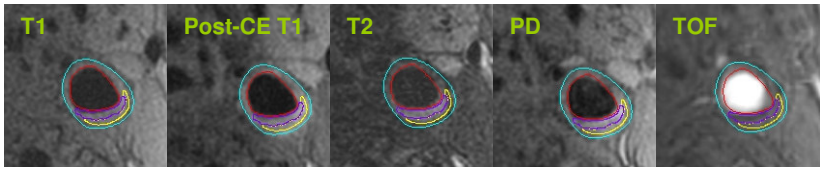


Figure 4.



and % hemorrhage. Additionally, the plaque integrity will also be evaluated and described as ruptured, thin, or thick fibrous cap, with and without ulceration, with and without thrombus.

VII. Statistical Analysis Methods Associated with Each of the Specific Aims

There are four "families" of analysis with internally similar statistical methods to be carried out under this grant. We describe each of the families of analysis in turn.

However, prior to more formal analysis we will explore the data to detect outliers or other distribution problems and display the data in graphical and tabular format using histograms, boxplots, scatterplots, frequency listings for dichotomous and categorical variables, and descriptive statistics for all patients and subcategories of patients. We will also calculate the various outcome measures, such as percent lipid in the carotid arterial wall, as a mean per MRI slice, in order to control for the varying number of MRI slices per subject. Throughout the analyses we will take account of potential differences in MRI measurements due to the effect of scan platform or study site. The site effect is a difference among measurements that can not be explained away by patient characteristics, such as age and gender, or study site. We can formally test for a site effect, describe its magnitude and adjust for it using random effects models. Similarly, the platform (fixed) effect can be addressed by using a dummy variable for the platform. The site and platform effects are confounded (because groups of sites use one of the two platforms). Even so, the site effect can be addressed by a model that nests the sites within platform.

A second issue is patient dropout and the potential bias introduced by dropout. Moderate dropout, such as that anticipated here (17% maximum) is not much of a problem unless it is very differential between the two treatment groups. We will compare demographics and all key baseline variables between dropouts and non-dropouts. In the unlikely event that the dropout rate is strongly associated with an important and statistically significant predictive variable, then we will adjust for that variable in the analysis

1. Treatment effect. The first set of analyses addresses the primary and secondary aims of determining the treatment effect on lipid composition, plaque wall volume and wall thickness (aims 1d and 2b). The simplest form of this analysis will be a t-test or nonparametric Mann-Whitney test comparing the two treatment groups on the change in an outcome variable between baseline and the 2-year MRI assessment. In order to control for important covariates, we will carry out multivariate linear regression, with the 2-year change in the outcome, such as percent of wall volume that is lipid-rich necrotic core, being the dependent variable and independent variables of treatment group (dichotomous), baseline value of the outcome variable, and any other variables that need to be controlled, such as age and gender. We can also test for an interaction between variables in affecting the outcome by using an interaction term in the model. We will not automatically test all possible interactions. If main effects are included in a final regression model, it is natural to test to see if there is an interaction between some of the main effects. Choice of which interactions to try will be driven by biological plausibility. For example, if treatment and baseline lipid composition are important and statistically significant main effects in a model, it is natural to test to see if their interaction is also important. In general, quite large sample sizes are needed to detect interactions, unless they are very strong. However, the sign and magnitude and even marginal statistical significance of an interaction are informative, even if the interaction term is not ultimately included in the model. This comment on use of statistical interaction terms applies to the regression analyses for all of our aims. We routinely carry out statistical diagnostics on these analyses, such as examining residuals.

2. Risk of cardiovascular events. A second set of analyses will focus on the risk of CV events in relation to plaque characteristics and the changes over time in the plaque. We will use methods of survival analysis (failure-time analysis) for this part of the study (aims 3b, 3c). We will use Kaplan-Meier plots, the log rank test and the Cox proportional hazards model for these analyses. The outcome variable is time to a first CV event during the study period, and those who do not have an event by the end of follow-up surveillance will be considered as censored. The two branches of this

endeavor are (i) the relation of plaque volume and characteristics at one cross-sectional moment in time (such as baseline) to the risk of a subsequent event; and, (ii) the relationship between plaque "velocity"—the rate of change of plaque characteristics over time—to subsequent risk of an event. The velocity and cross-sectional status of plaque are two quite different biological concepts, and it may happen that one or the other alone, and not both, are important. For the first analysis, (i), each patient will have up to two time intervals: baseline to an event or to the 2-year MRI assessment, and then from the 2-year MRI assessment forward to the end of follow-up. By using two intervals per person, one initiated at baseline and the other initiated at 2 years, we will always be using the most recent MRI data on plaque status to compare to subsequent follow-up. This analysis is carried out by using time-varying covariates (135). For the analysis of CV risk in relation to plaque "velocity", (ii), the methodology is the same, except that each person will have only one interval, from the 2-year MRI scan onward. Independent variables will be the rate of change of plaque characteristics during the first 2 years (e.g., annual % change in lipid composition), as well as the value of plaque variables at the 2-year point, a new "baseline". For both the baseline and velocity part of these analyses we will attempt to build a multivariate model(s) that will allow us to characterize the CV risk for a given patient. These models will use not only the MRI variables but currently identified risk factors as well, such as age and gender. Our modeling will also address an important issue: Do the plaque variables, even if statistically significant, really add to the prediction of cardiovascular risk? I.e., are the plaque variables really adding something independent of what is already known about risk? We can address this issue by comparing a Cox proportional hazards model with traditional risk factors only, such as age, gender, and smoking status, to a model that includes these traditional factors and also includes any important plaque variables that have detected. We can use either the change in a pseudo-R^2 statistic (based on likelihood) or use logistic regression and note the increase in the area under the ROC curve when the plaque variables are added into the model with the traditional risk factors. This comment applies to any analyses where there are traditional (known) risk factors available. While statistical significance of a plaque variable may help us to understand the biology better, it is also helpful to know if the use of plaque variables notably increases the prediction of CV events for individuals.

3. Correlation among baseline plaque, plaque changes and laboratory variables. A third set of analyses deal with correlation between baseline plaque characteristics and plaque progression or between laboratory measurements, such as lipids and inflammatory markers, and plaque composition and progression (aims 1c and 4c). The simplest form of these analyses will be the Pearson or Spearman correlation (and scatterplots) between pairs of variables, such as the change over two years in plaque lipid composition vs. the corresponding change in HDL cholesterol concentration. The correlations will be based on a pair of variables, one each selected from these separate sets of variables: (i) baseline plaque variables, (ii) baseline laboratory measurements, (iii) rate of change of plaque variables over time, (iv) rate of change of laboratory measurements over time. Each different pair of variables and their correlation test a hypothesis of interest. For example, selection of the change in plaque over time and change in a laboratory variable over time tests the hypothesis that these items progress or regress in parallel. These analyses are readily expanded to include covariates in multivariate models. For example, if baseline HDL cholesterol and baseline plaque lipid composition are both related to progression we would want to try including them as independent variables in a model for progression in order to tease out the independent effect of each of the two variables.

The study will be able to detect even rather weak correlations. With our sample size of at least 220 patients who have compete data for up to 2 years we will be very likely to

detect a true correlation of r = ±0.18 or larger between variables of interest. We are assuming 80% power, 2-sided test, $p<0.05$.

4. Description of changes over time. The final set of analyses are simply descriptive—documenting the changes that occur to plaque variables and laboratory measurements over time, from baseline through the first 2 years of treatment up to the second MRI (aim 4b). These results will be mean annual rates of change, along with a standard deviation (SD), standard error, confidence interval, and a statement of the statistical significance of the rate in comparison to a null hypothesis of a zero rate of change. These estimates of mean rates of change provide a succinct picture of changes, they are useful for planning of future studies, and they will be quite precise. Given the 220 patients and even a very highly variable measurement with a large SD for the 2-year change, say, 50% of the baseline value, the 95% confidence interval for the mean change will still have a width of only ±6% of the baseline mean, a very narrow interval. A more realistic example of a variable, with an SD for change of 25%, would have a very tight confidence interval of width ±3%. The study will also be able to detect even small departures from a zero rate of change over time. For example, If, again, 2 different variables have SDs of change over time of 25% and 50%, as just defined, then we are very likely to detect a true difference from a zero change over 2 years if the true change is at least 5% or 10%, respectively.

MAGNETIC RESONANCE (MR) PROCEDURE SCREENING FORM FOR PATIENTS

Date ____/____/____ Patient Number _____

Name _____ Age _____ Height _____ Weight _____
Last name First name Middle Initial

Date of Birth ____/____/____ Male ❑ Female ❑ Body Part to be Examined
month day year

Are you certain about if you have had prior surgery or injury of any kind? ❑ No ❑ Yes

If you are not certain you do not need to fill out the following

1. Have you had prior surgery or an operation (e.g., arthroscopy, endoscopy, etc.) of any kind? ❑ No ❑ Yes
   If yes, please indicate the date and type of surgery:
   Date ____/____/____ Type of surgery _____
   Date ____/____/____ Type of surgery _____
   Date ____/____/____ Type of surgery _____
   Date ____/____/____ Type of surgery _____
   Date ____/____/____ Type of surgery _____
   Date ____/____/____ Type of surgery _____
   Date ____/____/____ Type of surgery _____
2. Have you experienced any problem related to a previous MRI examination or MR procedure? ❑ No ❑ Yes
   If yes, please describe: _____
3. Have you had an injury to the eye involving a metallic object or fragment (e.g., metallic slivers, shavings, foreign body, etc.)? ❑ No ❑ Yes
   If yes, please describe: _____
4. Have you ever been injured by a metallic object or foreign body (e.g., BB, bullet, shrapnel, etc.)? ❑ No ❑ Yes
   If yes, please describe: _____
5. Are you currently taking or have you recently taken any medication or drug? ❑ No ❑ Yes
   If yes, please list: _____
6. Are you allergic to any medication? ❑ No ❑ Yes
   If yes, please list: _____
7. Do you have a history of asthma, allergic reaction, respiratory disease, or reaction to a contrast medium or dye used for an MRI, CT, or X-ray examination? ❑ No ❑ Yes
8. Do you have anemia or any disease(s) that affects your blood, a history of renal (kidney) disease, or seizures? ❑ No ❑ Yes
   If yes, please describe: _____

For female patients:

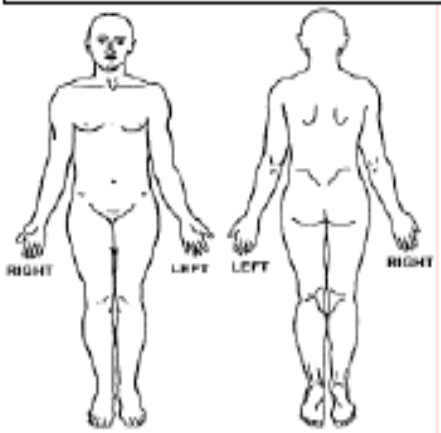
9. Post menopausal? ❑ No ❑ Yes
10. Are you pregnant or experiencing a late menstrual period? ❑ No ❑ Yes
11. Are you currently breastfeeding? ❑ No ❑ Yes

WARNING: Certain implants, devices, or objects may be hazardous to you and/or may interfere with the MR procedure (i.e., MRI, MR angiography, functional MRI, MR spectroscopy). Do not enter the MR system room or MR environment if you have any question or concern regarding an implant, device, or object. Consult the MRI Technologist or Radiologist BEFORE entering the MR system room. The MR system magnet is ALWAYS on.

Please indicate if you have any of the following:

- ☐ Yes ☐ No Aneurysm clip(s)
- ☐ Yes ☐ No Cardiac pacemaker
- ☐ Yes ☐ No Implanted cardioverter defibrillator (ICD)
- ☐ Yes ☐ No Electronic implant or device
- ☐ Yes ☐ No Magnetically-activated implant or device
- ☐ Yes ☐ No Neurostimulation system
- ☐ Yes ☐ No Spinal cord stimulator
- ☐ Yes ☐ No Internal electrodes or wires
- ☐ Yes ☐ No Bone growth/bone fusion stimulator
- ☐ Yes ☐ No Cochlear, otologic, or other ear implant
- ☐ Yes ☐ No Insulin or other infusion pump
- ☐ Yes ☐ No Implanted drug infusion device
- ☐ Yes ☐ No Any type of prosthesis (eye, penile, etc.)
- ☐ Yes ☐ No Heart valve prosthesis
- ☐ Yes ☐ No Eyelid spring or wire
- ☐ Yes ☐ No Artificial or prosthetic limb
- ☐ Yes ☐ No Metallic stent, filter, or coil
- ☐ Yes ☐ No Shunt (spinal or intraventricular)
- ☐ Yes ☐ No Vascular access port and/or catheter
- ☐ Yes ☐ No Radiation seeds or implants
- ☐ Yes ☐ No Swan-Ganz or thermodilution catheter
- ☐ Yes ☐ No Medication patch (Nicotine, Nitroglycerine)
- ☐ Yes ☐ No Any metallic fragment or foreign body (Shrapnel, bullet, BB pellet)
- ☐ Yes ☐ No Wire mesh implant
- ☐ Yes ☐ No Tissue expander (e.g., breast)
- ☐ Yes ☐ No Surgical staples, clips, or metallic sutures
- ☐ Yes ☐ No Joint replacement (hip, knee, etc.)
- ☐ Yes ☐ No Bone/joint pin, screw, nail, wire, plate, etc.
- ☐ Yes ☐ No IUD, diaphragm, or pessary
- ☐ Yes ☐ No Dentures or partial plates
- ☐ Yes ☐ No Tattoo or permanent makeup
- ☐ Yes ☐ No Body piercing jewelry
- ☐ Yes ☐ No Hearing aid *(Remove before entering MR system room)*
- ☐ Yes ☐ No Other implant _____
- ☐ Yes ☐ No Breathing problem or motion disorder
- ☐ Yes ☐ No Claustrophobia

Please mark on the figure(s) below the location of any implant or metal inside of or on your body.



IMPORTANT INSTRUCTIONS

Before entering the MR environment or MR system room, you must remove all metallic objects including hearing aids, dentures, partial plates, keys, beeper, cell phone, eyeglasses, hair pins, barrettes, jewelry, body piercing jewelry, watch, safety pins, paperclips, money clip, credit cards, bank cards, magnetic strip cards, coins, pens, pocket knife, nail clipper, tools, clothing with metal fasteners, & clothing with metallic threads. Please consult the MRI Technologist or Radiologist if you have any question or concern BEFORE you enter the MR system room.

NOTE: You may be advised or required to wear earplugs or other hearing protection during the MR procedure to prevent possible problems or hazards related to acoustic noise.

I attest that the above information is correct to the best of my knowledge. I read and understand the contents of this form and had the opportunity to ask questions regarding the information on this form and regarding the MR procedure that I am about to undergo.

Patient's signature: _____ Date ____/____/_____

MD/RN/RT signature: _____ Date ____/____/_____

Print MD/RN/RT name: _____

MAGNETIC RESONANCE (MR) ENVIRONMENT SCREENING FORM FOR INDIVIDUALS*

The MR system has a very strong magnetic field that may be hazardous to individuals entering the MR environment or MR system room if they have certain metallic, electronic, magnetic, or mechanical implants, devices, or objects. Therefore, all individuals are required to fill out this form BEFORE entering the MR environment or MR system room. Be advised, the MR system magnet is ALWAYS on.

*NOTE: If you are a patient preparing to undergo an MR examination, you are required to fill out a different form.

Date ____/____/____ (month / day / year) Name _____ (Last Name / First Name / Middle Initial) Age _____

Are you certain about if you have had prior surgery or injury of any kind? ☐ No ☐ Yes

If you are not certain you need not fill out the following

1. Have you had prior surgery or an operation (e.g., arthroscopy, endoscopy, etc.) of any kind? ☐ No ☐ Yes
   If yes, please indicate date and type of surgery: Date ____/____/____ Type of surgery_____
2. Have you had an injury to the eye involving a metallic object (e.g., metallic slivers, foreign body)? ☐ No ☐ Yes
   If yes, please describe: _____
3. Have you ever been injured by a metallic object or foreign body (e.g., BB, bullet, shrapnel, etc.)? ☐ No ☐ Yes
   If yes, please describe: _____
4. Are you pregnant or suspect that you are pregnant? ☐ No ☐ Yes

WARNING: Certain implants, devices, or objects may be hazardous to you in the MR environment or MR system room. Do not enter the MR environment or MR system room if you have any question or concern regarding an implant, device, or object.

Please indicate if you have any of the following:

- ☐ Yes ☐ No Aneurysm clip(s)
- ☐ Yes ☐ No Cardiac pacemaker
- ☐ Yes ☐ No Implanted cardioverter defibrillator (ICD)
- ☐ Yes ☐ No Electronic implant or device
- ☐ Yes ☐ No Magnetically-activated implant or device
- ☐ Yes ☐ No Neurostimulation system
- ☐ Yes ☐ No Spinal cord stimulator
- ☐ Yes ☐ No Cochlear implant or implanted hearing aid
- ☐ Yes ☐ No Insulin or infusion pump
- ☐ Yes ☐ No Implanted drug infusion device
- ☐ Yes ☐ No Any type of prosthesis or implant
- ☐ Yes ☐ No Artificial or prosthetic limb
- ☐ Yes ☐ No Any metallic fragment or foreign body
- ☐ Yes ☐ No Any external or internal metallic object
- ☐ Yes ☐ No Hearing aid *(Remove before entering the MR system room)*
- ☐ Yes ☐ No Other implant_____

IMPORTANT INSTRUCTIONS

Remove all metallic objects before entering the MR environment or MR system room including hearing aids, beeper, cell phone, keys, eyeglasses, hair pins, barrettes, jewelry (including body piercing jewelry), watch, safety pins, paperclips, money clip, credit cards, bank cards, magnetic strip cards, coins, pens, pocket knife, nail clipper, steel-toed boots/shoes, and tools. Loose metallic objects are especially prohibited in the MR system room and MR environment.

Please consult the MRI Technologist or Radiologist if you have any question or concern BEFORE you enter the MR system room.

I attest that the above information is correct to the best of my knowledge. I have read and understand the entire contents of this form and have had the opportunity to ask questions regarding the information on this form.

Patient's signature: _____ Date ____/____/____

MD/RN/RT signature: _____ Date ____/____/____

Print MD/RN/RT name: _____

HDL Proteomics Substudy

Appendix 6

The "Plaque Inflammation and Dysfunctional HDL in AIM-HIGH" (HDL Proteomics) Substudy

Principal Investigator:
Kevin D. O'Brien, MD
Co-Investigators:
William Kerwin, PhD;
Tomas Vaisar, PhD,
Xue-Qiao Zhao, MD;
Jeffrey L. Probstfield, MD

Funding Source/Dates:
1R01HL089504-01A1
05/01/2008 to 04/30/2013

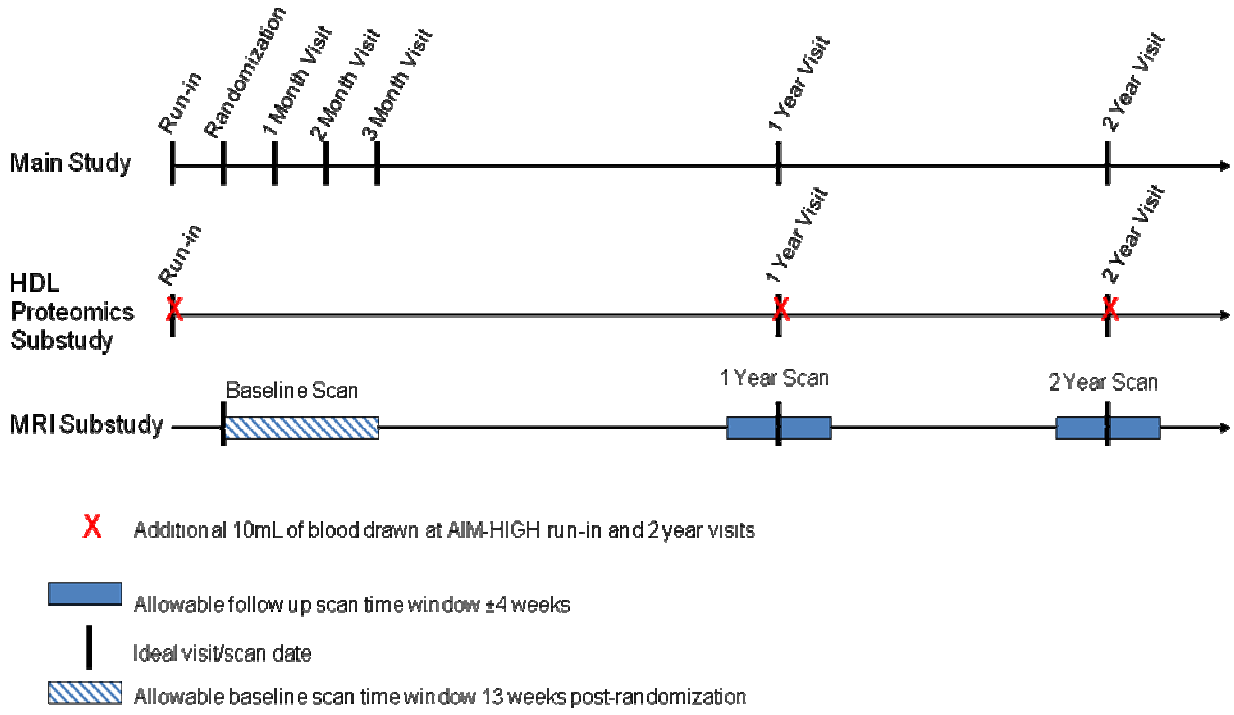
Synopsis: The overall goal of this proposal is to use state-of-the-art imaging and proteomic approaches to understand the roles of macrophages and HDL in preventing CHD in a subset of the unique participants available from the AIM-HIGH Trial. By utilizing the well-established AIM-HIGH trial recruitment, clinical site and data collection infrastructure, this Substudy will be much more efficient and cost-effective than would a stand-alone, multi-center trial. In preliminary studies, we have obtained preliminary evidence that CHD is characterized by oxidative and inflammatory changes in HDL that are associated with impairment of its normal function but that are improved with statin+niacin therapy. We also have found a strong correlation between a dynamic contrast-enhanced (DCE-MRI) parameter, K_{trans}, and plaque inflammation, and have identified low HDL levels as the clinical factor that correlates most strongly with K_{trans}. In this context, the AIM-HIGH cohort presents a unique opportunity to investigate the relative effects of simvastatin or simvastatin+niacin on specific inflammatory changes in atherosclerotic plaques.

Based on these findings, we propose a Substudy that will enroll participants concurrently with Xue-Qiao Zhao's Substudy of MR imaging in AIM-HIGH patients. We will measure plasma HDL oxidation and protein composition and will perform post-processing of MR images to derive parameters associated with carotid inflammation at baseline, and after 1 year and 2 years on either simvastatin or simvastatin+ niacin. In **Aim 1**, we will test the hypothesis that 2 years of simvastatin+niacin results in greater reduction in HDL oxidation and normalization of HDL protein composition than does simvastatin alone. In **Aim 2**, we will test the hypothesis that simvastatin+niacin results in greater reduction in the carotid inflammation marker, K_{trans}, at 2 years than does simvastatin alone. In **Aim 3**, we will test the hypothesis that HDL oxidation changes over 2 years correlate better with reduction in K_{trans} than do changes in HDL levels alone.

Thus, this Substudy will use novel, state-of-the-art, non-invasive imaging and protein analytical tools to determine whether niacin therapy in concert with a statin reduces plaque inflammation and dysfunctional HDL to a greater extent than does a statin alone. The results would provide strong support for the hypothesis that niacin-induced alterations in HDL are of central importance in decreasing atherosclerotic plaque inflammation.

I. Sub-study Overview

The "Plaque Inflammation and Dysfunctional HDL" (HDL Proteomics) Substudy will be carried out in parallel with the Main AIM-HIGH study and the Carotid MRI Substudy. A total of 200-300 subjects will be enrolled, and each will undergo three plasma sample collections: Run-In, Year 1 and Year 2. The HDL-Proteomics Sub-study will be carried out concurrently with Dr. Xue-Qiao Zhao's Carotid MRI Sub-study, and we anticipate that at least 120 individuals will be participants in both Sub-studies. This will allow 200-300 participants for HDL studies in Aim 1 and 120 participants for MRI studies in Aim 2 as well as for HDL/MRI comparisons in Aim 3. See the timeline in Figure 1 below:



THE PURPOSE of this sub-study is to examine the effect of intensive LDL-lowering plus HDL-raising therapy, compared with LDL-lowering alone, on: 1) HDL oxidation and protein composition, 2) atherosclerotic plaque inflammation in carotid arteries assessed by post-acquisition processing of multi-contrast MRI images obtained as a part of Dr. Xue-Qiao Zhao's Carotid MRI Sub-study and 3) the correlation between changes in HDL oxidation and plaque inflammation.

THE PRIMARY HDL ENDPOINTS are:

For HDL:

1) Change in HDL oxidation (3-chlorotyrosine levels) from Run-In to Year 2 for the whole cohort (simvastatin+niacin, S+N, and simvastatin, S) and between treatment groups (S+N vs. S).
2) Change in 5 HDL proteins (identified by shotgun proteomics, then quantified by multiple-reaction monitoring mass spectrometry) from Run-In to Year 2 for the whole cohort (S+N and S) and between treatment groups (S+N vs. S).

For MRI:

1) Change in a contrast-enhanced MRI marker of inflammation, K^{trans}, from Baseline to Year 2 in the whole cohort (S+N and S) and between treatment groups (S+N vs. S).

For HDL and MRI correlation:

1) Correlation between changes in HDL oxidation and K^{trans}, from Baseline to Year 2 in the whole cohort (S+N and S) and between treatment groups (S+N vs. S).

THE SECONDARY ENDPOINTS include correlation of HDL oxidation and protein content changes with changes in MRI-assessed plaque composition (lipid, volume, fibrous material, loose matrix, hemorrhage), as well as examining the time course of HDL oxidation and protein changes from Baseline through Years 1 and 2.

II. Subject Inclusion and Exclusion Criteria

1. Patients must qualify for and be enrolled in the main AIM-HIGH study (please refer to the parent study's *AIM-HIGH Manual of Operations* for inclusion and exclusion criteria).
2. Patients must be willing to participate and sign informed consent.

III. Clinical Procedures Coinciding with Run-In Plasma Sample Collection

DURING RUN-IN CLINICAL VISIT

1. **Identify potential subjects.** All patients who attend the run-in clinical visit and are willing to sign informed consent are eligible for the blood draw portion of this sub-study.
2. **Obtain informed consent.** Use your site's IRB approved consent form.
    a. The original signed record must be kept in the patient's research folder
    b. Provide a copy of the consent for the patient's personal records
3. **Draw additional 10 mL blood sample into EDTA-containing (purple top) tube.** An additional purple top tube will be drawn during the run-in visit. Once collected, spin blood and aliquot PLASMA into **four labeled 1.0 mL tubes** for freezing and overnight shipment on dry ice to the Northwest Lipid Research Lab.

IV. Clinical Procedures Coinciding with Year 1 Clinic Visit

DURING YEAR 1 CLINICAL VISIT

1. **Identify participating subjects.** Subjects who consented during their run-in visit and have not withdrawn consent will receive a follow-up Year 1 blood draw.
2. **Draw additional 10 mL blood sample into EDTA-containing (purple top) tube.** An additional purple top tube will be drawn during the run-in visit. Once collected, spin blood and aliquot PLASMA into **four labeled 1.0 mL tubes** for freezing and overnight shipment on dry ice to the Northwest Lipid Research Lab.

V. Clinical Procedures Coinciding with Year 2 Clinic Visit

DURING YEAR 2 CLINICAL VISIT

3. **Identify participating subjects.** Subjects who consented during their run-in visit and have not withdrawn consent will receive a follow-up Year 2 blood draw.
4. **Draw additional 10 mL blood sample into EDTA-containing (purple top) tube.** An additional purple top tube will be drawn during the run-in visit. Once collected, spin blood and aliquot PLASMA into **four labeled 1.0 mL tubes** for freezing and overnight shipment on dry ice to the Northwest Lipid Research Lab.