PROTOCOL SYNOPSIS

A Phase 2 Randomized, Double-Blind, Placebo-controlled Study to Evaluate the Effects of Two Doses of MEDI6012 on Apolipoprotein B100 Metabolism in Subjects with Stable Atherosclerotic Cardiovascular Disease.

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Version History:
Version 1. 3/2018
Version 2. 5/2018
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Version 4. 8/2018
Version 5. 9/2018
Version 6. 10/2018
Version 7. 11/2018
Version 8. 7/2019

Study site(s) and number of subjects planned

Columbia University Medical Center

622 West 168th Street, NY, NY 10032

This is a single site study that will enroll a maximum of 8 subjects.

Study period		Phase of development
Estimated date of first subject enrolled	12/15/2018	Phase 2
Estimated date of last subject completed	11/30/2019	

I. Study design

This is a Phase 2, single-center, placebo controlled, double-blind, randomized crossover study to determine the effects of MEDI6012 on the metabolism of apolipoprotein B100 (apoB100) lipoproteins in individuals with stable atherosclerotic cardiovascular disease (ASCVD). Studies will use stable isotope to determine the *in vivo* kinetics of apoB100 in VLDL, IDL, and LDL as well as VLDL TG. The primary outcome will be the percent difference in the fractional clearance rate (FCR) of LDL apoB100 on treatment with MEDI6012 versus on treatment with placebo. FCR is defined as the percent of the circulating pool of LDL apoB100 cleared from the plasma each day. At least 8 subjects will be randomized at Columbia University Medical Center to evaluate the effect of an IV push dosing regimen that includes a loading dose of 300 mg MEDI6012 followed by a 150 mg maintenance dose of MEDI6012 at 48 hours.

Once consented, subjects will undergo a screening period of up to 28±4 days. For subjects requiring a washout of dyslipidemia medication other than a statin or ezetimibe, or supplement, a 56±2day screening period is allowed (with repeat screening safety and lipids labs within 28±4 days of enrollment). Once enrolled and randomized, subjects will be instructed in an AHA heart healthy diet and stay on that diet, with frequent follow-up telephone calls to obtain diet histories and to reinforce the diet over two weeks before inpatient visit. They will then be admitted to the inpatient research unit of the Irving Institute for Clinical and Translational Research. On day 0, they will arrive in the morning and have fasting blood samples. The first dose of either active drug or placebo will be administered by IV push in the morning hours. They will be observed for the next 2 hrs. for immediate IP reactions and then discharged home with guidelines to continue to follow an AHA Heart Healthy Diet. They will return to our center at 12pm the next day (Day 1-post first dose) and be admitted to our unit for 2 nights. At the time of admission, stat safety bloods will be drawn to ensure no adverse effects from first IP administration. They will be provided with lunch and dinner but will be NPO after 8pm. At 1am (or 8 hrs before the stable isotope administrations) (Day 2) they will start liquid feedings (q2hrs) as part of a protocol obtaining a nutritional steady state before the start of stable isotope administration and kinetic studies. The composition of this liquid formula is 57% carbohydrate, 18% fat, and 25% protein. They will receive the 2nd dose of either active drug or placebo on the morning of Day 2 (exactly 48 hrs. from Day 0 administration). One hour after the second dose (Day 2-9am), a preisotope administration blood draw will be obtained (0hr). Immediately after blood draw stable isotopes will be administered to subjects for the study of lipid and lipoprotein kinetics, and 17 timed blood samples (see procedure schedule) will be obtained for the next 24 hr. followed by discharge. Subjects will repeat this same protocol 6-8 weeks later but will receive the alternative treatment. In the period between the first and second studies, subjects will be monitored and re–instructed as needed in the AHA diet. Two nutritional assessments via 24 hr. recalls will ensure that all subjects maintain a constant healthy lifestyle during the washout period between each study. All subjects will have a 'safety' visit 28±4 days after the first study period. A blood sample will be obtained to ensure that the subject's plasma lipid levels are the same as those during the first study. If they differ significantly, intensive diet and lifestyle counseling will be initiated and continued on a bi-weekly basis for the rest of the inter-study period.

Follow up, for safety monitoring, will also be performed 28±4days after the first and second study. ADA samples will be obtained at days 0 of each inpatient study, as well as 28±4days after the end of the study. If ADA present at day 28±4 days after first study subjects will be monitor after 56±4 days and will not continue to the second period (see subject replacement rules) of the study. If ADA present at day 28±4 days after second study period (active drug), an additional sample for ADA will be obtained at approximately day 56±4 days post study. For both periods, if a subject's Day 56±4 immunogenicity sample is confirmed as ADA positive and there is a > 30% decrease in HDL-C and/or presence of a neutralizing antibody, the subject will return to the study site approximately every 3 months thereafter for additional assessments until their immunogenicity sample is no longer ADA positive or their HDL-C has returned to baseline levels or until approximately 12 months after the 2nd dose of treatment was administered, whichever occurs first. Additional follow-up may be done if clinically/medically necessary.

II. Objectives

Primary objective: To compare the effects of two IV doses of MEDI6012 to two IV doses of placebo on the FCR of LDL-apoB100 (pools/day) in patients with stable atherosclerotic cardiovascular disease.

Secondary objective: To compare the effects of two IV doses of MEDI6012 to two doses of placebo on the production rate (PR) of LDL apoB100 (mg/kg/day) in patients with stable atherosclerotic cardiovascular disease.

To access the immunogenicity of MEDI6012 via measurement of ADA.

Exploratory Objectives: To assess the effects of IV doses of MEDI6012 on various parameters of lipoprotein metabolism and kinetics in plasma, including:

1. Plasma lipid panel parameters, including total cholesterol, high density lipoprotein cholesterol (HDL-C), TG, and calculated LDL cholesterol (LDL-C) (l determined by automated analyzer), free and esterified cholesterol determined by thin layer chromatography. Cholesterol (free and esterified) and triglyceride levels in isolated lipoprotein fractions (VLDL, IDL, LDL, and HDL) determined by automated analyzer.
2. ApoB100 concentration (ELISA) in plasma and in VLDL, IDL and LDL.
3. FCR of apoB100 in VLDL and IDL.
4. PRs of apoB100 in VLDL and IDL.

5. Conversion of VLDL to LDL.

6. Concentration of apoAI and apoAII in plasma and HDL fractions (ELISA).

7. Lipoprotein particle size and number profile via ion mobility.

8. LCAT Mass (concentrations) in serum.

9. Plasma sterol (campesterol, beta sitosterol, lathostherol) measurements by mass spectrometry.

10. CETP mass by ELISA.

11. Plasma concentrations of apoE and apo-CIII (ELISA).

III. TARGET SUBJECT POPULATION

Adult men or women aged 35 through 80 years with a history of documented stable atherosclerotic CVD. Subjects should be on stable statin dose for greater than 8 weeks. Some may also be on ezetimibe for at least 8 weeks. Women will be postmenopausal.

IV. INVESTIGATIONAL PRODUCT, DOSAGE, AND MODE OF ADMINISTRATION

300 mg of MEDI6012 on Day 0 (loading dose), a second dose of 150 mg 48 hours later (Day 2) – both by IV push over 1-2 minutes.

V. Noninvestigational medicinal product(s) Formulations:

2H3-L-leucine in sterile normal saline

L-phenylalanine-13C6 ring in sterile normal saline

1,1,2,3,3-$^{2}H_5$-glycerol **Routes of**

administration:

Bolus:

2H3-L-leucine

L-phenylalanine-13C6 ring

1,1,2,3,3-$^{2}H_5$-glycerol **IV**

infusion:

2H3-L-leucine administered via a constant IV infusion **Dose regimen:**

IV bolus:

2H3-L-leucine (10 µmol/kg)

L-phenylalanine-13C6 ring (29.4 µmol/kg)

[1,1,2,3,3-$^{2}H_{5}$]-glycerol (100 µmol/kg) IV

infusion:

2H3-L-leucine at 10 µmol/kg/h for 15 hours

VI. DURATION OF TREATMENT

Subjects will complete a one-day screening visit and one enrolment visit. Once inclusion and exclusion criteria are met, they will be scheduled for their inpatient stay (within 4 weeks/28±4 days of last safety bloods/screening visit). Subjects who require a washout of lipid treatments will have a screening period of 56±2 days. They will have a second screening visit within28±4 of their inpatient visit. There are two periods in this double-blind, randomized crossover study, separated by a 6-8

week wash out between treatments. Thus, subjects will be randomized to receive two doses of placebo or two doses of MEDI6012 at 0 and 48 hours during the first study period and two doses of the other drug during the second study period. Follow up, for safety monitoring, will be performed 28±4 days after the completion of each study period. ADA samples will be obtained at days 0 of inpatient kinetic outcome study and 28±4 days after each study period. If ADA is present at day 28±4days, a second sample will be obtained as soon as possible and if positive, the subject study order will be unblinded and if the subject was on active drug, the study will be stopped, and that subject will have an additional sample for ADA will obtained at day 56±4. Further follow-up for continued presence of ADA is described above on page. A new subject will be enrolled to replace the one with an incomplete study.

VII. STATISTICAL CONSIDERATIONS

Sample Size: This is an exploratory pilot study. We estimate, from prior studies, that administration of MEDI6012, on the background of high potency statin, will reduce LDL apoB (extrapolating from total apoB changes) by 10-15%. If we assume that there will be no change in LDL apoB PR, we can expect a somewhat larger effect size for LDL apoB FCR: about 15-20%. With 8 subjects studied on placebo and then on treatment, there will be statistical significance (p=0.012, two-tailed), by the nonparametric Wilcoxon signed rank test, if all 8 subjects increase their FCRs, regardless of the magnitude and inter-subject variability of the increases. By the parametric paired t-test, there will be statistical significance if the average increase in FCR is equal to or greater than the SD of the change (e.g., >=20% increase when SD=22%, >=15% increase when SD=17%). In our previous studies, the SD of percent change in paired studies has been smaller than the actual percent change. In other words, there will be statistical significance by a paired t-test if the effect size is 1 or greater, which has been the case in our prior studies.

Therefore, we plan to enroll and complete studies on 8 subjects. Drop-outs will be replaced, although we do not, based on a very long and large experience, expect this to occur.

Statistical Analyses: The primary PD endpoint will be the percent change (and 95% confidence limits) in the FCR of LDL apoB100 during administration of MEDI6012 from the FCR of LDL apoB100 during administration of placebo. The significance of this change will be assessed using either a Wilcoxon Signed Rank test. If the intra-individual variability of change is 17% or less, we can also use a paired t-test at the two-sided alpha level of 0.05.

All PD parameters, including concentrations of lipids and apolipoproteins in isolated lipoproteins, additional lipoprotein kinetic parameters, the concentrations of lipids and apolipoproteins in plasma, and the concentrations and sizes of lipoprotein particles will also be summarized as percentage change (and 95% confidence limits) on active drug versus placebo. Paired t-tests will be used to assess differences, but these will all be exploratory analyses. Plots of the mean and individual subject values will also be provided over time for selected parameters.

VIII. Safety

The safety analysis will be based on the review of descriptive statistics (summary tables) and individual data for adverse events (cases) and clinical laboratory tests. Adverse events will be coded using medical dictionary for regulatory activities (MEDRA), and the number of subjects with treatment-emergent adverse events (TEAEs) will be summarized. Potentially clinically significant abnormalities (PCSAs) for clinical laboratory, vital sign, and ECG data and out-of-normal range values for clinical laboratory data will be flagged and summarized in frequency tables. Results of ADA data will be summarized for each timepoint.

1. INTRODUCTION

1.1 Background

Atherosclerosis, the underlying condition of atherosclerotic cardiovascular disease (CVD), is a progressive condition associated with significant comorbidity and mortality. Excess cholesterol in arteries induces inflammation, decreases endothelium-dependent vasorelaxation, and promotes plaque instability (1-3). Periods of plaque instability can result in acute coronary syndrome (ACS), a spectrum of life-threatening clinical conditions that include unstable angina and non-ST- and STsegment elevation myocardial infarction (NSTEMI and STEMI).

Plaque rupture is caused by the dissolution of the fibrous cap, the dissolution itself being the result of the release of metalloproteinases (collagenases) from activated inflammatory cells. This event is followed by platelet activation and aggregation, activation of the coagulation pathway, and vasoconstriction. Treatment for ACS is therefore focused on drugs that rapidly inhibit platelet aggregation and/or blood clot formation (4); antiplatelet agents including aspirin and the adenosine diphosphate receptor antagonists clopidogrel, prasugrel, and ticagrelor, which can be given orally, together with the intravenously (IV) administered IIb/IIIa receptor antagonists abciximab, eptifibatide, and tirofiban. Commonly used anticoagulants include low-molecular weight heparins, thrombin inhibitors, and Factor Xa inhibitors. Drug therapies as well as percutaneous coronary interventions (PCI; balloon angioplasty and stent deployment) are focused only on the culprit lesion and do not adequately address the underlying cause of plaque vulnerability for rupture (i.e., cholesterol deposition) or reduce the risk of new plaque ruptures at other sites. While chronic lipid lowering therapy with statins reduces the risk of both primary and secondary cardiovascular (CV) events by lowering plasma low-density lipoprotein-cholesterol (LDL-C), it is not thought to acutely stabilize plaque. It has been postulated that a therapy that could rapidly remove plaque cholesterol would stabilize vulnerable plaques in ACS patients, reduce the likelihood of subsequent ischemic events, and address an important unmet medical need (5). Furthermore, this same mechanism would be expected to have similar results in the carotid and peripheral vasculature (6-8)

Lecithin: Cholesterol Acyltransferase: *LCAT,* is a 63 kDa size glycoprotein containing 416 amino acids that is secreted into the circulation by the liver where it primarily associates with HDL and to a lesser degree LDL (9). LCAT is believed to be a key enzyme in the Reverse Cholesterol Transport (RCT) process, the pathway by which excess cellular cholesterol is removed from peripheral cells by HDL and is delivered to the liver for excretion (10). LCAT catalyzes the conversion of cholesterol to cholesteryl esters (CE) on lipoproteins by the transacylation of fatty acid from the sn-2 position of phosphatidylcholine to the 3-hydroxyl group on the A-ring of cholesterol. Because of the increased hydrophobicity of CE compared to free cholesterol, CE formed by LCAT on the surface of lipoprotein particles partitions into the neutral lipid core of lipoproteins. The great majority of circulating CE in the plasma compartment (approximately 75%) is derived from the action of LCAT, with the remainder made by an intracellular enzyme called ACAT. The cholesterol esterification process by LCAT leads to the maturation of discoidal, nascent pre-beta HDL to spherical alpha-migrating forms of HDL. Because the smaller forms of HDL are more quickly catabolized, patients with low levels of LCAT have an overall marked decrease in HDL levels and likely less efficient RCT. The esterification of cholesterol on HDL also increases the concentration gradient for free cholesterol between cell membranes and lipoproteins in the extracellular fluid and thus has been proposed to increase cholesterol efflux from peripheral cells (11). The esterification of cholesterol by LCAT also traps cholesterol in the core of the lipoprotein particle, until it can be removed by hepatic receptors, such as SR-B1, for excretion into the bile.

Preclinical animal models have shown that transient increased expression of LCAT can in itself substantially raise HDL-cholesterol (HDL-C) and may therefore increase RCT (12, 13). In rabbits that express endogenous cholesteryl ester transfer protein (CETP), transgenic overexpression of human LCAT has been shown to protect against diet-induced atherosclerosis (14). Similarly, IV administration of recombinant rabbit LCAT (rate) to wild-type rabbits has also been shown to protect against atherosclerosis (15). Overexpression of LCAT in rabbit and monkey models shows up to a 10-fold increase in LCAT activity with a corresponding 2- to 5-fold increase in HDL-C with no adverse effects noted(16, 17).

Investigational Product Background (IB)

MEDI6012 is briefly described below. *Refer to the current Investigator's Brochure for more details.* MEDI6012 is recombinant human lecithin-cholesterol acyltransferase (rhLCAT), an approximately 60 kilodalton, glycosylated, single-chain protein consisting of 416 amino acids produced via Chinese hamster ovary cell culture. It is being explored as an acute treatment to reduce the risk of recurrent cardiovascular events as an adjunct to the standard of care in patients with acute myocardial infarction (MI). MEDI6012 and ACP501 have the identical amino acid sequence and are therefore considered the same molecular entity.

Summary of Clinical Experience

Two completed studies with MEDI6012 are summarized in the IB (D5780C00002 and D5780C00005). Relevant to the current proposal, **Study D5780C00005** was a Phase 2a study to evaluate the safety, PK, and PD of multiple doses of MEDI6012 in subjects with stable atherosclerotic cardiovascular disease. In this study, 32 subjects were administered multiple doses of MEDI6012 or placebo (6:2 ratio). MEDI6012 or placebo was administered three times weekly at 3 dose levels (1-hour IV infusion of 40, 120, or 300 mg on Days 1, 8, and 15) and a 4th cohort tested an IV push dosing regimen that included a loading dose of 300 mg followed by 150 mg second dose at 48 hours followed by a 100 mg maintenance dose 7 days later. The current proposal uses the 300mg loading dose followed by 150mg second dose at 48 hours and no maintenance dose.

Efficacy - After multiple doses of MEDI6012 in Study D5780C00005, the primary PD endpoints of sustainable and reversible dose-dependent response for HDL-C, HDL-CE and CE were met. In addition, the study demonstrated dose dependent, statistically significant increases in apoAI and statistically significant increase in LDL-C at the 120 and 300 mg dose levels with no increase in apoB100.

Safety - In study D5750C00005, multiple doses of MEDI6012 were generally well tolerated. There was one TESAE of atrial fibrillation felt due to external factors unrelated to Investigational Product or study procedures. There were no deaths, or life-threatening TEAEs or TEAEs leading to withdrawal from the study. Non-serious TEAEs were reported by 52% (13 of 25 of all MEDI6012-treated subjects and 43% (3 of 7) of all placebo-treated subjects. All TEAEs were of mild or moderate intensity. At the PT level, the most frequent TEAEs in all MEDI6012 subjects were dyspnea (3 subjects), injection site reaction (2 subjects), non-cardiac chest pain (2 subjects) and diarrhea (2 subjects). There were no clinically relevant changes in vital signs, ECG, or standard safety laboratory parameters. No subject treated with MEDI6012 tested positive for treatment-emergent ADA.

1.2 Rationale for study design and doses

The premise of MEDI6012 clinical development is that the administration of MEDI6012 in patients with acute MI will increase HDL-C and confer cardio protection by upregulating mobilization of cholesterol from tissues, including cholesterol from atherosclerotic plaques in coronary, cerebrovascular, and peripheral arteries. This will result in stabilization of atherosclerotic plaques and a consequent decreased risk for recurrent major adverse CV events. In addition, expected improvements in HDL function may result in the modulation of inflammation and improvements in endothelial function, effects that may contribute to a reduction in major adverse CV events.

Previous studies with MEDI6012 administered at various doses have shown acceptable safety profile and dose-dependent increases in HDL-C, HDL-CE, and CE (see IB). However, in addition to the lipid changes above, levels of LDL-C were increased by 15%, with no changes, or actual decreases in LDL apoB100 levels of about 10-15%. The current proposal aims to define the mechanisms responsible for LDL-C and LDL- apoB changes.

1.2.1 LDC-C increases:

In the case of rhLCAT administration, if LDL cholesterol levels increase when someone receives rhLCAT, there are several possible mechanisms that can be considered, including:

a. An increase in VLDL-apoB100 secretion (production rate) into the circulation with greater generation of LDL-apoB100 via conversion of VLDL to LDL.

b. No change in VLDL-apoB secretion but either an increase in conversion of L to LDL (less direct removal of VLDL remnants) or a decrease in the FCR of LDL apoB100.No changes in either the production of LDL-apoB particles or the FCR of LDL-apoB100 but rather an increase in the size and cholesterol ester content of LDL (same number of particles but more cholesterol per particle).

In the first 2 instances, one would expect that the increase in LDL cholesterol would be accompanied by a similar increase in LDL apoB100 levels. In the last instance, one might see no change in apoB100 or, if the efficiency of LDL particles actually increased, a decrease in LDL apoB100 levels. No change or an increase in the efficiency of LDL clearance, as determined by the FCR, with larger more cholesterol ester enriched LDL particle, would translate to greater delivery of cholesterol to the liver per day (increased reverse cholesterol transport).

1.2.2 LDL-ApoB decreases:

As noted above, if LDL-C levels increase with no change or decreases in LDL apoB100 levels, this would mean more efficient delivery of LDL cholesterol to the liver. Current Medimmune data in humans actually show modest decreases in LDL apoB100 concentrations suggesting just that scenario: increased clearance of LDL particles with more CE per LDL particle.

These findings suggest that the generation of CE in HDL by rhLCAT, together with CETP activity, increases LDL cholesterol and, either by directly altering the affinity of LDL for the LDL receptor or indirectly increasing hepatic LDL receptors, leads to increase efficiency of removal of LDL particles. Thus, despite an increase in LDL cholesterol (the balance of enrichment of particles with CE and the same or increased LDL FCR), there is an increase delivery of circulating CE to the liver (RCT) with rhLCAT treatment.

This current Phase 2 study is designed to provide the mechanisms whereby rLCAT treatment changes LDL-apoB100 levels. The subjects participating in this study will have established atherosclerosis in at least one vascular bed (coronary, carotid, or peripheral arteries) and be on chronic statin therapy (with or without additional ezetimibe treatment). Subjects will be given two doses of MEDI6012 and two doses of placebo as stated above. Subjects may see transient changes in lipid/lipoprotein parameters with administration of active drug but are not expected to derive durable therapeutic benefit as they will receive only 2 doses of the drug. Subject risk will be minimized through strict eligibility criteria to avoid enrolment of unstable or high-risk subjects and by close monitoring of adverse events (AEs), laboratory parameters, and vital signs during the study. In addition, immunogenicity will be evaluated on an ongoing basis over the course of the study.

1.3 Research Hypotheses

1.3.1 Primary Hypotheses

Repeat dosing with MEDI6012 will increase the FCR of LDL-apoB100 lipoproteins.

1.3.2 Secondary Hypotheses

Repeat dosing with MEDI6012 will result in no change in LDL apoB100 PR.

2. OBJECTIVES

2.1 Objectives

2.1.1 Primary Objective

To compare the effects of two IV doses of MEDI6012 to placebo on the FCR of apoB100 in pools/day in patients with stable atherosclerotic cardiovascular disease on stable statin therapy.

2.1.2 Secondary Objectives

To compare the effects of two IV doses of MEDI6012 to placebo on the PR of LDL apoB100, in mg/kg/day in patients with stable atherosclerotic cardiovascular disease on stable statin therapy.

To evaluate the safety and immunogenicity of MEDI6012

2.1.3 Exploratory Objectives

a. To determine the effects of MEDI6012 on the levels of plasma total cholesterol, triglycerides, LDL and HDL.

b. To determine the effects of MEDI6012 on the levels of cholesterol and TG in isolated lipoprotein fractions (VLDL, IDL, LDL, HDL) as well as levels of apoB100 in plasma, VLDL, IDL, and LDL.

c. To determine the effects of MEDI6012 on the FCR of apob100 in VLDL and in IDL; the PR of apob100 in VLDL and IDL; the conversion of VLDL to LDL.

d. To determine the effects of MEDI6012 on free and esterified cholesterol.

e. To determine the effects of MEDI6012 on the concentrations of apoAI and apoAII in plasma and HDL fractions.

f. To determine the effects of MEDI6012 on lipoprotein particle size and number profile.

g. To determine the effects of MEDI6012 on LCAT concentrations in serum.

h. To determine the effects of MEDI6012 on lathosterol, campesterol, and beta sitosterol levels in plasma.

i. To determine the effects of MEDI6012 on CETP mass in serum.

j. To determine the effects of MEDI6012 on the concentrations of apoE and apoCIII in serum.

3. Study Endpoints

3.1. Primary Endpoint

Determination of the percent change in the FCR of LDL apoB100 during treatment with MEDI6012 compared to treatment with placebo via analysis of lipoprotein kinetic test parameters.

3.2. Secondary Endpoints

Determination of the percent change in the PR of LDL apoB100 during treatment with MEDI6012 compared to treatment with placebo via analysis of lipoprotein kinetic test parameters. We will also examine development of ADA.

3.3 Exploratory Endpoint

a. Plasma levels of total cholesterol, HDL-C, TG, calculated LDL cholesterol (LDL-C) and, ApoB100 measured via automatic analyzer in mg/dl.

b. Cholesterol and triglyceride levels in lipoprotein fractions (VLDL, IDL, LDL and HDL) via automatic analyzer in mg/dl.

C. apob100 concentration (via ELISA) in plasma and in VLDL, IDL and LDL isolated fractions (mg/dl).

d. Free and esterified cholesterol measured at three times points during each study period via TLC.

e. Lipoprotein Kinetic Test

- The FCR of apoB100 in VLDL, IDL (mg/kg/day)
- The PR apoB100 in VLDL, IDL (pools/day)
- the conversion of VLDL to LDL

f. the concentration of apoAI and apoAII in plasma and HDL fractions (ELISA) in mg/dl.

g. Lipoprotein particle size and number profile via ion mobility.

h. LCAT Mass (concentrations) in serum.

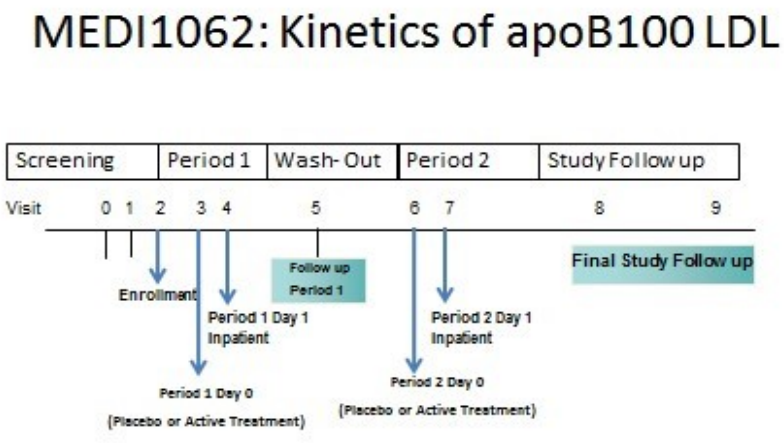
i. Plasma sterol (campesterol, beta sitosterol, lathostherol) measurements by mass spectrometry.

j. CETP mass by ELISA.

k. Plasma concentration apolipoprotein E and Apolipoprotein CIII via ELISA.

4. STUDY DESIGN

4.1 Overview



4.2 Description of the Study

This is a Phase 2, single-center, placebo controlled, double-blind, 2-period, and 2-treatment study to determine the effects of MEDI6012 on the kinetics of apoB100-lipoproteins in individuals with stable atherosclerotic cardiovascular disease (ASCVD). Studies will use stable isotope to determine the *in vivo* kinetics of apoB100 in VLDL, IDL, and LDL. The primary outcome will be the percent difference in the FCR of LDL apoB100 on treatment with MEDI6012 versus on treatment with placebo. At least 6 subjects will be randomized at the Columbia University Medical Center to evaluate the effect of an IV push dosing regimen that includes a loading dose of 300 mg MEDI6012 followed by a 150 mg maintenance dose of MEDI6012 at 48 hours.

Subjects will undergo a screening period of up to 28±4 days. For subjects requiring a washout of dyslipidemia medication other than a statin or ezetimibe, or supplement, a 56±2 days screening period is allowed (with repeat screening safety and lipids labs within 28±4 days of enrollment). Once enrolled and randomized, subjects will be instructed in an AHA heart healthy diet and stay on that diet, with frequent follow-up telephone calls to obtain diet histories and to reinforce the diet. They will

Then be admitted to the inpatient research unit of the Irving Institute for Clinical and Translational Research. On day 0, they will arrive in the morning hours and have fasting blood samples (see schedule of events Appendix, Table 1). The first dose of placebo/MEDI6012 will be administered by IV push in the morning. They will be observed for the next 1-2 hrs. for immediate IP reactions and then discharged home with guidelines to continue to follow an AHA Heart Healthy Diet (18). They will return to our center at 12pm the next day (Day 1-post first dose) and be admitted to our unit for 2 nights. At the time of admission, stat safety bloods will be drawn to ensure no adverse effects from first IP administration. They will be provided with lunch and dinner but will be NPO after 8pm. At 1am (Day 2- or 8 hrs before the start of stable isotopes) they will start liquid feedings (q2hrs) as part of obtaining a steady state (diet) before the start of the stable isotope administration and kinetic studies. The composition of this liquid formula is 57% carbohydrate, 18% fat, and 25% protein. They will receive the 2nd dose of placebo/MEDI6012 on the morning of Day 2 (exactly 48 hrs. from Day 0 administration). One hour after the 2nd dose, a pre-stable isotope blood draw will be performed (0hr). Immediately after, stable isotopes will be administered to subjects for the study of lipid and lipoprotein kinetics, and 17 timed blood samples will be obtained for the next 24 hr. followed by discharge. Subjects will repeat this same protocol 6-8 weeks later with alternate treatment from first period. In the period between the first and second studies, subjects will be monitored and re–instructed as needed in the AHA diet. We will ensure that all subjects to maintain a constant healthy lifestyle during the washout period between each study.

Follow up, for safety monitoring, will be performed 28±4 days after the second study. ADA samples will be obtained at days 0 of each study period inpatient visit, as well as 28±4 days after each study period. If ADA present at day 28±4 after study period, an additional sample for ADA will be obtained at approximately day 56. If a subject's Day 56±4 immunogenicity sample is confirmed as ADA positive and there is a > 30% decrease in HDL-C and/or presence of a neutralizing antibody, the subject will return to the study site approximately every 3 months thereafter for additional assessments (TABLE 1) until their immunogenicity sample is no longer ADA positive or their HDL-C has returned to baseline levels until approximately 12 months after the 2nd dose of treatment was administered, whichever occurs first. Additional follow-up may be done if medically necessary.

4.3 Treatment Regimen

Enrollment of 8 subjects is planned to evaluate the effects of a 300 mg loading dose of MEDI6012, followed by a 150 mg maintenance dose of MEDI6012 48 hours later, on lipid and lipoprotein metabolism.

4.4 Dose Rationale

PD observations from the single-ascending dose study of MEDI6012 (D5780C00002) and Cohorts 1 and 2 from the second study (D5780C00005) demonstrated that the rate of increase of HDL-C and apoAI are dose dependent. For future studies in ACS and acute MI subjects, maximizing the rate of increase of HDL-C and apoAI following the first and second

dose, may allow a coupling of the antiatherosclerotic effects of enhanced reverse cholesterol transport with other cardioprotective effects of HDL-C and apoAI resulting in multiple benefits for CHD patients (19-22). Therefore, the above dose regimen will be used for this proposal. The loading dose and maintenance doses have been selected based on PK/PD analysis that integrated PK/PD data from the single-ascending dose study of MEDI6012 (D5780C00002) and PD data from study (D5780C00005). Simulations utilizing the RCT PK/PD model were performed based on the estimated PK/PD parameters to select doses in this study that can characterize MEDI6012 PK and the range of PD effects when administering MEDI6012 with loading and maintenance doses administered via IV push (see IB).

In summary, the proposed dose regimen of MEDI6012 is expected to be well tolerated, and the collected PK/PD data will be appropriate to fulfill the objectives of this study. The follow-up duration of 28±4 days after dosing is deemed appropriate to evaluate the reversibility of potential safety findings and to characterize the potential immunogenicity of MEDI6012 when serum concentration (PK mass) has completely cleared and PD biomarkers return to baseline values. Additional follow-up beyond the initial 28±4 days is appropriate in ADA positive subjects with a concomitant decrease in HDL-C or presence of a neutralizing antibody to ensure an ADA does not have a detrimental effect on endogenous LCAT, a theoretic risk of MEDI6012.

4.5 Rationale for Study Population

The study population consists of adults with a history of documented atherosclerotic CVD, including individuals with significant coronary artery calcium on CT scanning, who are clinically stable on current lipid lowering therapy with a statin or statin plus ezetimibe. This population is similar to the study population evaluated in the single ascending dose study (stable coronary heart disease) and multiple ascending dose study (stable atherosclerotic CVD) of MEDI6012.

This population strikes the best balance to permit safety, PK, and PD assessment of MEDI6012 in subjects with established atherosclerosis, the target population for subsequent clinical development, but who are clinically stable (lower safety risk) with less fluctuation in biomarker levels to enable robust PK/PD decisions. Subjects with unstable atherosclerotic CVD will be excluded. Subjects will be required to be on a stable dose of statin or statin plus ezetimibe regimen as is the standard of care for atherosclerotic disease, with LDL-C levels ≤ 120 mg/dL at screening. This requirement is to avoid enrolling subjects with genetically low LDL receptor concentration (and thus high or very high baseline LDL-C) and to provide a more homogeneous population against which to evaluate the lipid/lipoprotein changes of interest.

Similarly, subjects with high baseline HDL-C values (> 70 mg/dL for men, > 80 mg/dL for women) will be excluded to provide consistency among the study subjects for the upward movement of HDL-C levels.

5. MATERIALS AND METHODS

5.1 **Subjects:** At least 8 subjects will be enrolled.

5.2 Inclusion Criteria

a. Adult male and female subjects (non-childbearing potential for females) ages 35 through 80 years at the time of screening who are capable of providing informed consent prior to screening and any protocol-related procedures.

b. Written informed consent and any locally required authorization (e.g., Health Insurance Portability and Accountability Act [HIPAA] in the USA) obtained from the subject prior to performing any protocol-related procedures, including screening evaluations.

c. Ability to complete and meet all eligibility requirements for randomization within 28 days of informed consent (56 days if washing out from lipid altering agent other than statins or ezetimibe).

d. A diagnosis of stable atherosclerotic CVD documented prior to screening:

- Coronary artery disease defined as a history of prior myocardial infarction, coronary revascularization, history of coronary atherosclerosis based on invasive or non-invasive imaging, and/or abnormal stress testing diagnostic of CAD.
- Carotid artery disease (extracranial ICA stenosis) defined as evidence of carotid atherosclerosis by carotid imaging, or history of percutaneous or surgical carotid revascularization
- Peripheral artery disease defined as ankle-brachial index < 0.90 and claudication, or prior peripheral revascularization for ischemia, or evidence of lower extremity (below the inguinal ligament) atherosclerosis on invasive or noninvasive imaging

e. Currently receiving statin as standard of care, at a stable dose for ≥ 8 weeks prior to screening and intended to remain at a stable dose throughout the study duration. Subjects may also be receiving ezetimibe, 10 mg/day for ≥ 8 weeks prior to screening.

f. Nonsterilized males who are sexually active with a female partner of childbearing potential must use condom and spermicide from Day 1 through the end of their participation in the study. Because male condom and spermicide is not a highly effective contraception method it is strongly recommended that female partners of a male study subjects also use a highly effective method of contraception throughout this period.

Highly Effective Methods of Contraception

- Tubal occlusion
- Copper T intrauterine device
- Levonorgesterel-releasing intrauterine system (e.g., Mirena®)
- Medroxyprogesterone injections (e.g., Depo-Provera®)

- Etonogestrel implants (e.g., Implanon®, Norplan®)
- Combined pills
- Norelgestromin/ethinyl estradiol transdermal system (e.g., Ortho Evra®)
- Intravaginal device (e.g., NuvaRing®)
- Cerazette® pill

5.3 Exclusion Criteria

a. Unstable cardiovascular conditions within 3 months prior to screening, including ACS, stroke or transient ischemia attack, critical limb ischemia, non-elective arterial revascularization, lifethreatening arrhythmias, or heart failure hospitalization.

b. Elective arterial revascularization (in any vascular territory) in the past 1 month. Any planned arterial revascularization (coronary, peripheral or carotid).

c. New York Heart Association (NYHA) Class III or IV congestive heart failure or treatment with advanced therapies (cardiac transplant, ventricular assist device, cardiac resynchronization therapy, and/or chronic IV inotropic support), or severe valvular heart disease.

d. Body mass index < 18 or > 45.

e. Lipid measurements with any of the following:
   1. TG > 400 mg/dL
   2. LDL-C > 120 mg/dL
   3. HDL-C > 70 mg/dL for males or > 80 mg/dL for females

f. Clinically significant vital sign abnormalities at screening or on Day -1:
   1. Systolic blood pressure (BP) < 90 or >160 mm Hg
   2. Diastolic BP > 100 mm Hg

g. Females currently breastfeeding or of childbearing potential. (Females of childbearing potential are defined as those who are not surgically sterile (i.e., bilateral tubal ligation, bilateral oophorectomy, or complete hysterectomy) or those who are not postmenopausal (defined as 12 months with no menses without an alternative medical cause and a follicle-stimulating hormone level in the central laboratory's normal range for post-menopausal phase is required at screening).

h. Use of lipid-lowering medications, with the exception of statins and ezetimibe, and the following dietary supplements: ≥ 2 grams/day of fish oil (≥ 2 grams/day DHA and EPA combined), ≥ 30 grams/day of flaxseed oil or ground flaxseed, red yeast extract, > 100 mg/day of niacin. (At the investigator's discretion, subjects may

undergo a 6-week washout period of any exclusionary lipid-lowering agents with the expectation that post-washout lipid levels will be rechecked and acceptable per above criteria)

i. History of any of the following:

- Documented familial hypercholesterolemia
- Chronic kidney disease defined by estimated glomerular filtration rate < 30 mL/min/1.73 m2 by the modification of diet in renal disease equation, or end stage renal disease treated with kidney transplant or renal replacement therapy
- History of clinically overt chronic liver disease or biochemical evidence of liver disease (e.g., AST or ALT >2.0 × ULN and/or total bilirubin > ULN (unless due to Gilbert's syndrome)
- Poorly controlled endocrine disorder including:
  -- Type 1 Diabetes excluded
  -- Type 2 Diabetes Mellitus with glycated hemoglobin [HbA1c] > 8.0% as assessed at screening or
- Uncontrolled thyroid disorder defined as thyroid stimulating hormone (TSH)> ULN and abnormal free T4; subjects with thyroid deficiency should have received a stable dose of thyroid hormone for > 6 weeks prior to screening and have a normal TSH.
- Current or previous use of systemic corticosteroids within 28 days prior to screening.
  Topical, intra-articular, intranasal, inhaled, and ophthalmic steroid therapies are allowed
- History of severe infection or ongoing febrile illness within 30 days of screening, or a medical history of a chronic viral illness including hepatitis B or C virus, or human immunodeficiency virus (HIV).
- History of active malignancy within 5 years (subjects with non-melanotic skin cancer may be included)
- Any other disease or condition or laboratory value that, in the opinion of the investigator or medical monitor, would place the subject at an unacceptable risk or interfere with the evaluation of the investigative product. **Note: for abnormal lab values, subjects may be re-assessed one time only if, in the investigator's judgment, the values are not representative of the subject; any further re-test beyond this must be discussed with the medical monitor in advance.**
- Known allergy/hypersensitivity to any component of the investigational product formulation, other biologics, IV infusion equipment, plastics, adhesive or silicone, history of infusion site reactions with IV administration of other medicines, or ongoing clinically important allergy/hypersensitivity as judged by the investigator.

j. Subjects who are legally institutionalized

An employee or close relative of an employee of the sponsor or the study site, regardless of the employee's role.

k. Previous Exposure to rhLCAT

l. Concurrent enrollment in another clinical study of any investigational drug therapy or use of any biologicals within 6 months prior to screening or within 5 half-lives of an investigational agent or biologic, whichever is longer. If the screening assessments are not considered to be representative of the usual status of the subject's health by the investigator, or if one or more exclusion criteria are considered temporary or from a reversible condition, repeat screening assessments to establish eligibility will be permitted on one occasion, at the discretion of the investigator.

5.4 Subject Enrollment and Randomization

Study participation begins,a subject is “enrolled”,once written informed consent is obtained. Once informed consent is obtained, a subject screening identification (SID) number will be assigned by the study site. Ex. LCAT101**S**. Once subject is enrolled (meets all the inclusion and exclusion criteria) in the study, a study ID will be assigned (Ex. LCAT101**E**).

The numbers will be used to identify the subject throughout study participation. A master log of all consented subjects will be maintained at the site and will document all screening failures (i.e., subjects who are consented but do not meet study eligibility criteria and/or are not randomized), including the reason(s) for screening failure.

Subjects who fail to meet the inclusion/exclusion criteria (i.e., screening failures) will not be randomized or receive investigational product. If a subject fails screening because of a clinically significant finding, they will be notified and provided with a report that they can take to their physician. The investigators will follow-up with such individuals to ensure that they have seen a physician regarding the clinically significant finding.

5.5 Withdrawal from the Study

Subjects are at any time free to stop study treatment or completely withdraw from the study (investigational product and assessments), without prejudice to further treatment. If complete withdrawal of consent is established, then no further study visits or data collection should take place. However, subjects who wish to discontinue investigational product only are expected to remain in the study for the follow-up of AEs and vital status.

Subjects who receive any amount of investigational product will be followed for protocol-specified assessments including follow-up of any AEs unless it is confirmed that consent is withdrawn. If a subject that received at least one

dose of investigational product becomes lost to follow-up, starts an alternative treatment, or is enrolled in another clinical trial, then at least vital status will be assessed at the end of the trial.

5.6 Discontinuation of Investigational Product

The investigator and/or medical monitor may determine that an individual subject will not receive any further investigational product if any of the following occur in the subject in question:

1. Safety reasons as judged by the investigator where continued treatment may put patient at undue risk, any other AE that in opinion of the investigator contraindicates further dosing.
2. Severe adverse immune reaction (e.g., anaphylactic-type reactions, immune complex disease) deemed related to investigational product.
3. Infusion site reaction assessed as intolerable and related to investigational product.
4. Incorrectly randomized patient in whom inclusion/exclusion criteria violation would put the patient at undue risk.
5. Subject noncompliance that, in the opinion of the investigator or Sponsor, warrants treatment discontinuation for safety reasons (e.g., refusal or inability to adhere to scheduled visits).
6. Pregnancy in any female subject.

5.7 Replacement of Subjects

Subjects who withdraw from the study or do not complete the kinetics studies (phase 1 and phase 2) must be replaced by the study PI to ensure that the data collected are sufficient to measure the study main outcomes. Although positive ADA have not been reported to date, if subject has positive results during study period 1, that subject will not continue period 2 of the study. The subject will be followed for safety and ADA levels. A new subject will be enrolled to replace the individual whose study was stopped for development of ADA in period 1.

5.8 Withdrawal of Informed Consent

Biological Samples Obtained for the Main Study

Study data are protected by the use of a SID (study identification number) number, which is a number specific to the subject. The investigator is in control of the information that is needed to connect a study sample to a subject. A subject's consent to the use of data does not have a specific expiration date, but the subject may withdraw consent at any time by notifying the investigator.

Samples Obtained for Future Research

Samples obtained for future research may be used for potential future analysis according to the subject's permission in the Consent Form. Samples obtained for future research will be labeled with a sample identification number but will not be labeled with personal identifiers such as the subject's name. A file linking this sample identification number with the SID number will be kept in a secure place by the study PI with restricted access. If the subject withdraws consent for participating in the future research, this link will allow the PI to locate the subject's sample and destroy it.

The coding of samples and results is to ensure that these research results are kept confidential by keeping the subject's identity and these results separate. If the subject consents to have his/her samples used for future research, this additional research may not start immediately and may start at any time during the storage period. The subject's sample(s) will be aliquoted and stored by the PI. The subject's samples will not be kept for more than 10 years after the end of the study in which they were collected.

If the subject chooses not to allow his/her study samples to be used for future research, the samples will be destroyed by the study site (PI) once they are no longer required for the main study.

If consent is withdrawn after a sample has been taken, but before the subject's sample is stored for future research, the investigator will arrange to have it destroyed.

If consent is withdrawn after the subject's sample(s) have been stored for future research, the investigator will ensure that these sample(s) are destroyed unless the identification number has been removed and the subject can no longer be linked to any sample(s). However, if the subject's samples have already been used for research, the PI is not required to destroy results of this research. In this case only the remaining sample(s) will be destroyed.

6. SCHEDULE OF STUDY PROCEDURES

See Appendix, Table 1.

6.1 Unscheduled Visits and Early Termination Visits

Depending on the purpose of the visit and discussion with the study team, the additional procedures may be performed at unscheduled visits and for subjects who terminate prematurely from the study including, but not limited to the following:

- Targeted physical examination
- Vital signs
- ECG
- Serum chemistry and hematology
- Urinalysis (including microalbumin)
- PD blood sample for key lipid and lipoprotein biomarkers (HDL-C, TC, TG, LDL-C)
- ADA/neutralizing antibody blood sample
- Assessment of AEs/SAEs
- Concomitant medications

6.2 Description of Study Procedures

6.2.1. Medical History, Physical Examination, Electrocardiograms, Weight, and Vital Signs

Medical History and Physical Examination

Medical history at screening will include history and current medical conditions, past or present CV disorders, respiratory, gastrointestinal, renal, hepatic, neurological, endocrine, lymphatic, hematologic, immunologic, dermatologic, psychiatric, genitourinary, drug and surgical history, or any other diseases or disorders.

A full physical examination will be conducted at screening, Day 0 or Day 1 (admission to inpatient facilities) of both study periods. We will also conduct a targeted physical exam at Day 28±4 post 2nd treatment.

Any focal deficit identified at baseline should be documented in the case report form (CRF). Clinically significant abnormal findings will be recorded at baseline and follow-up.

Height and body weight will be measured. Height will only be measured at the screening visit.
Weights will be measured as per schedule of events table.

Vital Signs

Vital sign measurements (BP, respiratory rate, heart rate, and body temperature) will be obtained after the subject has rested in the supine position for at least 5 minutes. They will be collected at screening, and every day during inpatient

stay. Measurements will be performed before and after IP and placebo administrations and as stated in study procedures Table

1.

Oral body temperature measurement will be taken according to local SOP.

The use of automated devices for measuring BP and heart rate is acceptable, although when done manually, BP should be taken in the upper arm, and heart rate must be measured in the brachial/radial artery for at least 30 seconds. Subjects must remain in the same position, supine or semi-recumbent, for the entire measurement and should be consistent for the entire study.

All BP determinations will be performed using calibrated and appropriately maintained equipment and will be used on the same subject throughout the study as much as possible. The same size blood pressure cuff, that has been properly sized and calibrated, will be used to measure BP each time.
Subject's arm must be at the same height (at the level of their heart) during each BP measurement.

At screening, BP should be measured on 2 consecutive occasions, once on each arm, to determine which arm potentially has the higher reading. Measurement from a single arm is acceptable when only one arm is accessible. This same arm should be used, whenever possible, for each BP assessment.

For time points where ECG recording precedes vital sign measurement, the 10-minute rest in the supine (or semi-recumbent) position prior to the ECG suffices for the rest prior to vital sign measurement.

Electrocardiograms

Electrocardiography will be used in this study to screen for significant abnormalities that suggest active cardiac disease. One ECG will be obtained at the screening visit.

ECGs will be recorded after the subject has rested for at least 10 minutes in the supine position. For subjects who are not able to remain supine, semi-recumbent position within 45 degrees of supine is allowed. Subjects must remain in the same position, supine or semi-recumbent, for the entire measurement and should be consistent for the entire study.

6.2.2. Clinical Laboratory Tests

All screening laboratory test will be performed at the CUMC hospital laboratory. (certificate will be provided and file in study SOP). A detailed SOP for sample collection, processing, storage, and shipment of samples, as well as laboratory contact information, specific to this clinical research study will be filed in study laboratory binder located in the Co-PI's laboratory.

Clinical laboratory safety tests will be performed in a licensed local clinical laboratory. Only postmenopausal women will be enrolled in this study. Clinically significant (as determined by the investigator) abnormal laboratory results should be repeated as soon as possible (preferably within 24 to 48 hours).

The following clinical laboratory tests will be performed:

Serum Chemistry

Calcium, chloride, potassium, sodium, bicarbonate, blood urea nitrogen, creatinine, glucose, albumin, CK, ALT, AST, ALP, total bilirubin

Aspartate transaminase (AST) • Alanine transaminase (ALT), Alkaline phosphatase (ALP), total bilirubin (will be assessed concurrently).

Hematology

- White blood cell count with differential (% and absolute counts)
- Platelet count
- Red blood cell count • Mean corpuscular volume

- Hematocrit • Mean corpuscular hemoglobin concentration
- Hemoglobin

Urinalysis (screening)

- Color • Blood
- Appearance • Leukocyte esterase
- Specific gravity • Bilirubin
- pH • Urobilinogen
- Protein • Nitrite
- Glucose • Urine microscopy and urine casts (as required)
- Ketones • Microalbumin

Coagulation Markers (screening)

Prothrombin Time (PT) and Partial Thromboplastin Time (PTT) will be measured by standard methods at CUMC Hospital laboratory.

Lipids for Eligibility (screening only, fasting for a minimum of 12 hours)-CUMC hospital Lab

- total cholesterol
- TG
- HDL-C
- calculated LDL-C

 Lipids for other visits will be processed in the IICTR Biomarkers laboratory (section 5.2.4).

Other Safety Tests (screening only)

- Thyroid stimulating hormone (at screening only), including T4 as applicable
- HbA1c (at screening only)
- Follicle-stimulating hormone (females only) in combination with 12 months of amenorrhea.

6.2.3. Pharmacokinetic Evaluation and Methods

The PK sampling (mass and activity) times and windows are detailed in the schedule of procedures in the appendix. Sampling within the specified window around the specified time will not be considered a protocol deviation but the exact time of sampling should be recorded. The concentration of MEDI6012 will be determined by immunoassay, Pacific Biomarkers (https://pacbio.com/biomarker/assaydetail/128/).

6.2.4. Pharmacodynamic Evaluation and Methods

All lipid levels will be measured as stated in the appendix via study site standard operating procedures. The following lipids and plasma lipoproteins are measured in a Cobas Integra 400 plus analyzer using Roche Diagnostic Systems and reagents. • Total Cholesterol, TG, HDL-C, Calculated LDL-C, ApoAI, apoAII.

Blood sample for other apolipoprotein biomarkers

For research purposes, exploratory endpoints of the following will be measured by validated ELISA kits. ApoB100 (MABTECH-3715-1HP-2) Mabtech, Inc. M.E.B. Suite 220, 3814 West Street, OH 45227 Cincinnati, United States. ApoE (Human Apolipoprotein E ELISA Kit (APOE) (ab108813), apoCIII (ab154131) ; 1 Kendall Square, Suite B2304, Cambridge, MA 02139-1517, USA, and, lipoprotein size and particle number for HDL, LDL, and VLDL may be characterized by ion mobility (23). Free and esterified cholesterol will be measured via TLC. CETP mass will measured by ELISA. Sterol measurements will be performed in the laboratory of Dr. Alice Lichtenstein (Tufts University) via mass spectrometry.

6.2.5. Kinetic Assessment methods

Lipoprotein Kinetics Test

Lipoprotein Kinetics Tests will be conducted while subjects reside in the inpatient research unit for 2 days (2 nights).

Subjects will be admitted to the inpatient research unit of the Irving Institute for Clinical and Translational Research. On day 0, they will arrive by 7:30am and have fasting blood samples. The first dose of placebo or MEDI6012 will be administered by IV push at 8am. They will be observed for the next 1-2 hrs. for immediate IP reactions and then discharged home with guidelines to continue to follow an AHA Heart Healthy Diet. They will return to our center at 12pm the next day (Day 1-post first dose) and be admitted to our unit for 2 nights. At the time of admission, stat safety bloods will be drawn to ensure no adverse effects from first IP administration. They will be provided with lunch and dinner but will be NPO after 8pm. At 1am (Day 2) they will start liquid feedings (q2hrs) as part of obtaining a steady state (diet) before the start of the stable isotope administration and kinetic studies. The composition of this liquid formula is 57% carbohydrate, 18% fat, and 25% protein. They will receive 16 feedings, which will provide 100% of their caloric needs, for 32 hours. The subjects will not consume any other foods, except the formula, during this period. Non-caloric, caffeine-free liquids will be allowed at this time and during the kinetic study.

At 6am on Day 2-two IV catheters will be placed in antecubital or forearm veins (one in each arm). Baseline blood samples will be drawn before the stable isotope administration (0hr baseline), and the actual kinetic study will commence at approximately 9 AM (1 hr. after 2nd dose administration) just after consuming a portion of the liquid formula and after administration of the second dose of placebo or MEDI6012. Through one catheter, subjects will receive an IV bolus of nutrient compounds labeled with stable isotopes: 2H3-L-leucine, L-phenylalanine-13C6 ring, and 2H5-glycerol (Cambridge Isotope Laboratories, Andover, MA), followed (through the same catheter) by a constant IV infusion of 2H3-Lleucine for the next 15 hours. The catheter used for the bolus injections and the infusion will be removed after completion of the infusion. Aseptic technique and methods suitable for the preparation and administration of IV solutions will be carefully observed. Serial blood samples will be taken frequently from the second catheter over the next 24 hours (10,20,40min,

1,2,4,6,8,10,12,14,15,15.5,16,18,21 and 24 hr.). After the 24-hour sampling, subjects will be counseled by the nutrition team on sensible diet for the next day, given breakfast and discharged.

Phase 2/Treatment Period 2 will begin 6-8 weeks after the last day of phase 1. There will be one follow up visit at day 28±4 between phase 1 and phase 2.

Isolation of Lipoproteins

VLDL, IDL LDL and HDL will be isolated from the plasma by sequential ultracentrifugation at the study center using standard methods (12) (19). The quantity (in mg/dL) of TG, cholesterol and apoB100 will be measured in VLDL, IDL, and LDL, and TG and cholesterol will be measured in HDL. ApoB in each lipoprotein will be separated from other proteins in each lipoprotein by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE). The band for each protein will be cut out of the gel and then hydrolyzed and derivatized (12) (20). Enrichment with 2H3-leucine and 13Cphenylalanine will be determined by the local laboratory using gas chromatography and mass spectrometry (GC/MS). The enrichment of plasma free leucine and phenylalanine will also be similarly determined. Apo B kinetics in VLDL, IDL, and LDL will be analyzed using both 2H3-leucine and 13Cphenylalanine enrichment data. Similar analyses will be performed to examine VLDL-TG kinetics using 2H5- glycerol enrichment data. PR and FCR values for apoB100 and TG will be derived from those values. The clinical research unit will follow its internal validated procedures for specimen collection, processing and storage.

The clinical research unit will follow its internal validated procedures for specimen collection, processing and storage, and these written procedures will be made available for use by local personnel during the study.

Plasma Lipid Panel

Blood samples for plasma lipid panels will be collected in the morning, in fasting condition (at least a 12-hour fast) at visits shown in the study flow chart. Alcohol consumption within 48 hours and intense physical exercise within 24 hours preceding blood sampling will be discouraged. If the subject is not fasting as required at a visit, he/she will be asked to return on the following day for the blood sampling, emphasizing compliance with the fasting requirement and the allowed time window for the visit.

Total cholesterol, HDL-C, TG, calculated LDL-C, and apoB100 will be assayed using well-established commercial assay methods in an accredited clinical laboratory. The analytical systems and reagents used to measure total cholesterol (an enzymatic method) and HDL-C (homogenous enzymatic method) must be certified by the Cholesterol Reference Method Laboratory Network (CRMLN) as having documented traceability to the national reference system for total cholesterol and the designated comparison method for HDL-C. The laboratory must be actively participating in a national proficiency testing program for total cholesterol and TG. The Friedewald formula will be used for calculating LDL-C in serum or plasma (LDL-C = TC – HDL-C – TG/5) (no one will be enrolled if their
TG ≥500 mg/dl).

6.2.6 Lipoprotein Particle Size Profile

At the same time points as for the Plasma Lipid Panel, plasma will also be analyzed for particle concentrations of VLDL, IDL, LDL and HDL sub fractions (nmol/L) using ion mobility (IM) methodology, which uniquely allows for direct particle quantification as a function of particle diameter following a procedure to remove other plasma proteins (28). The IM instrument utilizes an electrospray to create an aerosol of particles, which then pass through a differential mobility analyzer coupled to a particle counter. Particle concentrations in plasma (nmol/L) will be determined for VLDL, IDL, LDL, and HDL and their sub fractions as defined by discrete size (nanometer) intervals for VLDL (large, medium and small), IDL (large and small); LDL (large [LDL1], medium [LDL2a and LDL2b], small [LDL3a and LDL3b], and very small [LDL4a and LDL4b]; HDL (large [HDL2b]; smaller [HDL2a+3]).

7. INVESTIGATIONAL PRODUCT

7.1 Identification of Investigational Product

MedImmune will provide the investigator(s) with investigational product using designated distribution centers.

Identification of Investigational Products

Investigational Product	Manufacturer	Concentration and Formulation as Supplied
MEDI6012	MedImmune	Lyophilized powder (100 mg per mL upon reconstitution with sWFI) in a buffer consisting of 10 mM sodium phosphate, 300 mM sucrose, 0.06% (w/v) poloxamer188 at pH 7.2.
Placebo	MedImmune	10 mL of a solution containing 10 mM sodium phosphate, 300 mM sucrose, 0.06% (w/v) poloxamer188 at pH 7.2.

sWFI = sterile water for injection; w/v = weight by volume

MEDI6012 is provided as a sterile white to off-white lyophilized powder (50 mg/vial, nominal). Upon reconstitution with 0.6 mL sterile water for injection (sWFI), MEDI6012 is a colorless to yellow solution.

Placebo is provided as a sterile colorless to slightly yellow solution.

7.2 Investigational Product Handling

MEDI6012 and placebo must be stored at 2°C to 8°C (36°F to 46°F) at all times unless they are being used for dose preparation.

- MEDI6012 drug product requires reconstitution prior to use. Use a 1 mL syringe with 19G ½" needle (equivalent syringe and needle may be substituted for reconstitution).

- The reconstitution should be performed with 0.6 mL sWFI for each vial, with the liquid added gently to the side of the vial so the liquid stream is not directly added to the lyophilized cake. Commercially available sWFI may be supplied and should be stored at room temperature.

- The vial should be gently rotated or swirled until all the solids are dissolved. Gently invert the vial to dissolve any solids that may be present at the neck of the vial or on the stopper. The vial should not be shaken or vigorously agitated. The reconstituted product should appear as a colorless to yellow and clear to opalescent solution. A thin layer of bubbles on the liquid surface is considered normal.

Each IV push dose will be delivered as reconstituted MEDI6012 or placebo with a syringe and an IV administration set with 0.2 micron in-line filter. IV lines should be flushed with normal saline prior to drug administration. Each dose will be administered over 1-2 minutes and will be followed by a 10 mL normal saline flush.

7.3 Investigational Product Inspection

Each vial selected for dose preparation should be inspected. MEDI6012 is supplied as a sterile white to off-white lyophilized powder at a concentration of 100 mg/mL (50 mg/vial).

If there are any defects noted with the investigational product, the Investigator and Site Monitor should be notified immediately.

7.4 Dose Preparation Steps

MEDI6012 and placebo does not contain preservatives and any unused portion must be discarded. Preparation of investigational product is to be performed aseptically. Total in-use storage time from reconstitution of investigational product/needle puncture of the investigational product vial to start of administration should not exceed 4 hours at room temperature or 24 hours at 2°C to 8°C (36°F to 46°F). If prepared dose is stored at 2 to 8°C (36°F to 46°F), equilibrate to room temperature and inspect prior to IV administration to ensure solution is clear. If storage time exceeds these limits, a new dose must be

prepared using new vials. Only remove from storage the required MEDI6012 or placebo vials required for subject dosing.

The following table describes the delivery volume (mL), the required number of vials, and the number of syringes required for each dose.

MEDI6012 and Placebo Delivery Volume and Vial Usage

Dose (mg)	Total Delivery Volume (mL)		Number of Vials Required		Syringe Required for Administration	
	MEDI6012	Placebo	MEDI6012	Placebo	MEDI6012	Placebo
300 (Dose 1)	3	3	6	1	3 mL syringe	3 mL syringe
150 (Dose 2)	1.5	1.5	3	1	3 mL syringe	3 mL syringe

MEDI6012 or placebo may be pooled in each syringe (3 mL, polycarbonate) and doses prepared based on delivery volume. The injection will be administered over 1-2 minutes and followed by a 10 mL normal saline flush.

8. ASSESSMENT OF SAFETY

8.1 Systemic/Local Tolerability

Site staff will check the injection site(s) for local reactions and assess for systemic reactions at the times specified in the study procedures. Local and systemic reactions will be recorded as AEs.

Definition of Adverse Events will follow The ICH Guideline for Good Clinical Practice defines an AE as:

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. An AE includes but is not limited to any clinically significant worsening of a subject's pre-existing condition. An abnormal laboratory finding (including ECG finding) that requires medical intervention by the investigator, or a finding judged by the investigator as medically significant should be reported as an AE. If clinical sequelae are associated with a laboratory abnormality, the diagnosis or medical condition should be reported (e.g., renal failure, hematuria) not the laboratory abnormality (e.g., elevated creatinine, urine red blood cells increased). Abnormal

laboratory values that are not, in the investigator's opinion, medically significant and do not require intervention should not be reported as AEs. AEs may be treatment emergent (i.e., occurring after initial receipt of investigational product) or non-treatment emergent. A non-treatmentemergent AE is any new sign or symptom, disease, or other untoward medical event that begins after written informed consent has been obtained but before the subject has received investigational product. Elective treatment, surgery or preplanned treatment (that was scheduled prior to the subject being enrolled into the study) for a documented pre-existing condition that did not worsen from baseline is not considered an AE (serious or non-serious). An untoward medical event occurring during the prescheduled elective procedure or routinely scheduled treatment should be recorded as an AE or serious adverse event (SAE).

8.2 Definition of Serious Adverse Events:

An SAE is any AE that:

- Results in death
- Is immediately life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect in offspring of the subject
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above

Medical or scientific judgment should be exercised in deciding whether expedited reporting is appropriate in this situation. Examples of medically important events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalizations; or development of drug dependency or drug abuse.

8.3 Recording of Adverse Events

AEs will be recorded on the CRF using a recognized medical term or diagnosis that accurately reflects the event. AEs will be assessed by the principal investigator for severity, relationship to the investigational product, possible etiologies, and whether the event meets criteria of an SAE and therefore requires immediate notification to MedImmune.

If an AE evolves into a condition that meets the regulatory definition of "serious," it will be reported on the SAE Report Form. Infusion of biological products is commonly associated with infusion related reactions. Anaphylaxis and infusion-related reactions have some common manifestations and may be difficult to distinguish from each other. Infusion-related reactions are commonly observed during or shortly after the first time of exposure to biological products delivered through IV infusion. These reactions are less common following subsequent exposures. Unlike infusion-related reactions, anaphylaxis is a rare event, usually occurring after subsequent exposure to an antigen, and it is most commonly accompanied by severe systemic, skin and/or mucosal reactions. The investigator is advised to carefully examine symptoms of adverse reactions observed during or shortly after exposure to MEDI6012 and consider the above-mentioned facts prior to making a final diagnosis. Reactions occurring at the time of or shortly after subsequent infusions of investigational product are to be judged by the investigator at his/her own discretion.

8.4 Time Period for Collection of Adverse Events

Adverse events will be collected from time of signature of informed consent throughout the treatment period until end of Day 86 (FU1) or Day 114 (FU2). For ADA/neutralizing Ab positive, SAE collection will be continued until end of the follow-up period(s), with a maximum of 12 months from study active treatment administration.

8.5 Follow-up of Unresolved Adverse Events

Any AEs that are unresolved at the subject's last visit in the study are followed up by the investigator for as long as medically indicated, but without further recording in the CRF. MedImmune retains the right to request additional information for any subject with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

8.6 Reporting of Serious Adverse Events

All SAEs have to be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). All SAEs will be recorded in the CRF. All SAEs are to be submitted to the AstraZeneca Product Safety mailbox: AEMailboxClinicalTrialTCS@astrazeneca.com The investigator

ensures that all the necessary information is provided to the AstraZeneca Product Safety mailbox **within 24 hours calendar day** of initial receipt for fatal and life-threatening events and **within 5 calendar days** of initial receipt for all other SAEs. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

8.7 Other Events Requiring Immediate Reporting

8.7.1 Overdose

An overdose is defined as a subject receiving a dose of investigational product in excess of the maximum dose specified by the clinical study protocol.

- An overdose with associated AEs will be recorded as an AE diagnosis/symptom An overdose without associated symptoms will only reported on the CRF.
- If an overdose with a MedImmune investigational product occurs during the course of the study, then the investigator or other site personnel inform appropriate sponsor representatives immediately, or no later than 24 hours after becoming aware of the event. The designated sponsor representative works with the investigator to ensure that all relevant information is provided to the sponsor's patient safety data entry site. For overdoses associated with an SAE, the standard reporting timelines apply.

8.7.2 Hepatic Function

Abnormality Cases where a subject shows elevation in liver biochemistry may require further evaluation and occurrences of AST or ALT ≥ 3× ULN together with total bilirubin ≥ 2× ULN may need to be reported as SAEs. Refer to Appendix for further instruction on cases of increases in liver biochemistry and evaluation of Hy's Law.

8.7.3 Pregnancy

Women of childbearing potential are not allowed to be included in this study. Only Post-menopausal women will be included.

8.7.4 Paternal Exposure

Male subjects should refrain from fathering a child or donating sperm during the study and for 6 weeks following the last dose of investigational product.

Pregnancy of the subject's partners is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth or congenital abnormality), occurring from the date of the first dose until 6 weeks after the last dose should, if possible, be followed up and documented.

9. STUDY AND DATA MANAGEMENT

9.1 Training of Study Site Personnel

Before the first subject is entered into the study, all study staff (RA, NP, lab techs, study nurses, phlebotomist), will be trained in study protocol by the PI and co-investigator. They will review and discuss the requirements of the clinical study protocol and related documents with the investigational staff and also train them in any study-specific procedures and system(s) utilized.

The Principal Investigator will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information relevant to the performance of this study is forwarded to the staff involved.

The Principal Investigator will maintain a record of all individuals involved in the study (medical, nursing and other staff).

9.2 Monitoring of the Study

During the study, the sponsor of the study (Columbia University) and the study PI and co-PI will be responsible for the following:

- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, that data are being accurately and timely recorded in the CRFs, that biological samples are handled in accordance with the Laboratory Manual.
- Verifying that the research pharmacy keeps study drug accountability

- Perform source data verification including verification of informed consent of participating subjects.
- Ensure withdrawal of informed consent to the use of the subject's biological samples is reported and biological samples are identified and disposed of/destroyed accordingly, and the action is documented, and reported to the subject.
- An outside physician not involved in the research project will serve as safety officer.

9.3 Source Data

All source documents for the study will be created and stored by the study PI and Co-PI in the co-PI's office and laboratory space.

9.4 Study Agreements

The Principal Investigator will comply with all the terms, conditions, and obligations of the Clinical Study Agreement, or equivalent, for this study. In the event of any inconsistency between this clinical study protocol and the Clinical Study Agreement, the terms of clinical study protocol shall prevail with respect to the conduct of the study and the treatment of subjects and in all other respects, not relating to study conduct or treatment of subjects, the terms of the Clinical Study Agreement shall prevail.

9.5 Archiving of Study Documents

The investigator follows the principles outlined in the Clinical Study Agreement. CUMC does not provide long term storage of blood samples or source documents. Medimmune will need to provide long term storage for these after 1 year of study close out.

9.6 Study Timetable and End of Study

An individual subject will be considered to have completed the study if the subject completed both period 1 and period 2 outcome measurements.

Subjects will be considered not to have completed the study if consent was withdrawn or the subject was lost to follow-up.

The end of the study ("study completion") is defined as the date of the last protocol-specified visit/assessment for the last subject in the study (i.e., the follow up visit(s) after period 2 have been completed). If one or more subjects are determined to be ADA positive, then study completion will be defined as the date of the last visit/assessment for the last ADA-positive subject.

9.7 Data Management

CUMC will be responsible and accountable for the data management of this study according to the following:

a. All study data will be collected to paper CRF's.
b. Baseline characteristics will be entered by study personnel into a local electronic file developed by the study team.
c. The files are maintained by a local data management person and query as needed.
d. The investigator will ensure that data are recorded on the CRFs as specified in the study protocol and in accordance with the instructions provided.
e. The investigator ensures the accuracy, completeness, and timeliness of the data recorded according to the Clinical Study Agreement.
f. The investigator will sign the completed CRFs.

10. ETHICAL AND REGULATORY REQUIREMENTS

10.1 Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, and applicable regulatory requirements.

10.2 Subject Data Protection

The Informed Consent Form will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.

10.3 Ethics and Regulatory Review

The CUMC IRB will approve the final study protocol, including the final version of the Informed Consent Form and any other written information and/or materials to be provided to the subjects. The investigator will ensure the distribution of these documents to the applicable IRB, and to the study site staff.

The opinion of the IRB should be given in writing. The investigator will verify that all documents are approved and file copies of all approved (stamped) documents in the study regulatory binder before the start of the study.

The IRB will approve all advertising used to recruit subjects for the study. All/Any study modification should be submitted to the IRB and approved. All study document and protocols will require reapproval by the CUMC IRB annually.

MedImmune will provide the study site with safety updates/reports according to local requirements, including suspected unexpected serious adverse reactions (SUSARs), where relevant.

Each Principal Investigator is responsible for providing the IRB with reports of any serious and unexpected adverse drug reactions from any other study conducted with the investigational product. MedImmune will provide this information to the Principal Investigator so that he/she can meet these reporting requirements.

10.4 Informed Consent

The Principal Investigator(s) will:

- Ensure each subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study
- Ensure each subject is notified that they are free to discontinue from the study at any time
- Ensure that each subject is given the opportunity to ask questions and allowed time to consider the information provided
- Ensure each subject provides signed and dated informed consent before conducting any procedure specifically for the study
- Ensure the original, signed Informed Consent Form(s) is/are stored in the Investigator's Study File
- Ensure a copy of the signed Informed Consent Form is given to the subject

- Ensure that any incentives for subjects who participate in the study as well as any provisions for subjects harmed as a consequence of study participation are described in the informed consent form that is approved by an IRB

10.5 Changes to the Protocol and Informed Consent Form

Study procedures will not be changed without the mutual agreement of the site (study PI) and MedImmune. If there are any substantial changes to the study protocol, then these changes will be documented in a study protocol amendment and where required in a new version of the study protocol. The amendment is to be approved by the relevant IRB before implementation.

If a protocol amendment requires a change to a site's Informed Consent Form, MedImmune and the site's IRB are to approve the revised Informed Consent Form before the revised form is used.

If local regulations require, any administrative change will be communicated to or approved by each IRB.

10.6 Audits and Inspections

Authorized representatives of MedImmune, a regulatory authority, or an IRB may perform audits or inspections at the center, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice, guidelines of the ICH, and any applicable regulatory requirements. The investigator will contact MedImmune immediately if contacted by a regulatory agency about an inspection at the site.

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