Statistical Analysis Plan

for

VA Cooperative Clinical Trial Award Study #0002

Plaque Regression and Progenitor Cell Mobilization with Intensive Lipid Elimination Regimen

(PREMIER)

Version 2.0

September 30, 2014

CCTA 0002 PREMIER Study
Statistical Analysis Plan Version 2.0

Primary Safety Outcome Measure

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

Secondary Safety Outcome Measure

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

Primary Effectiveness Outcome Measure

The primary effectiveness outcome measure will be the change in the total atheroma volume within a ≥ 20 mm long segment of the target coronary artery from baseline to 12 weeks post-PCI. The measurement will be done via IVUS-VH at 2 time points (baseline during index PCI and 90-day follow-up).

Secondary Effectiveness Outcome Measures

The secondary effectiveness outcome measures will include:

1. The %NC component of atheroma. The %NC component of atheroma will be obtained via IVUS-VH at 2 time points (baseline during index PCI and 90-day follow-up).
2. EPC-CFU/ml of peripheral blood. The cell culture assay and quantification of circulating EPC-CFU will be performed for patients recruited at the Dallas VA center only. The assay will be done at 4 time points (pre-PCI, post-PCI, 30-day follow-up, and 90-day follow-up).

3. The major adverse cardiovascular endpoints (MACE) including death, myocardial infarction, coronary revascularization, and stroke during the follow-up periods. The major adverse CV events will be collected via both clinical visits for up to 6 months post-PCI and searches of the VA Austin database for up to 1 year post-PCI. Approvals for using the patients' Social Security Numbers for data searching will be obtained via informed consent form.

Sample Size Determination

There is only one study which examined the treatment of medication only (20mg Pravastatin or 10mg Simvastatin) vs. LDL-apheresis with statin medication on coronary plaque regression in familial hypercholesterolemia and published in 2002 Journal of American College of Cardiology[31]. The trial reported the net change of plaque area from baseline to one year follow-up. The changes were -0.69±2.08mm^2 in the LDL-apheresis group vs. 0.88±1.75mm^2 in the medication only group. Two studies, which have primarily mobilized coronary atheroma LDL using HDL or its apoprotein, have shown similar magnitude of atheroma regression at 6-10 weeks, without directly lowering LDL levels. Given the expected 85% reduction in LDL after LDL-apheresis, we expect to observe a similar treatment effect for the primary effectiveness outcome of this study at 12 weeks.

The sample size of 30 patients for the first feasibility/pilot study leads to a power of 54% with $\alpha = 0.05$ and the same effect size as reported in the published paper (Cohen's D effect size = 0.82 for -0.69 ± 2.08 mm^2 in the LDL-A group vs. 0.88 ± 1.75 mm^2 in the medication only group). The power decreases to 38% with $\alpha = 0.05$ and a more meaningful lower Cohen's D effect size of 0.65 by decreasing the difference of two treatment means by 20% (-0.38 ± 2.08 mm^2 in the LDL-A group vs. 0.88 ± 1.75 mm^2 in the medication only group). The power decreases further to 31% by considering 20% drop-out rate with $\alpha = 0.05$ and the Cohen's D effect size = 0.65.

The second stage of pivotal study is approved to obtain the 90% power to detect a Cohen's D effect size of 0.65 (-0.38 ± 2.08 mm^2 in the LDL-A group vs. 0.88 ± 1.75 mm^2 in the medication only group) with the randomization scheme of 1:1, $\alpha = 0.05$, and a 20% drop-out rate, the pivotal study needs a sample size of 128.

Baseline Characteristic Comparisons

Patient characteristics and outcome measures at baseline will be compared between the two treatment groups (ILLT vs. SMT) to determine if the treatment arms differ on any important variables. The chi-square test or Fisher's exact test will be used for categorical variables and the t-test for two independent samples will be used for continuous variables as shown in the following sample table:

	ILLT		SMT		
Variable	N	Mean±SD (or %)	N	Mean±SD (or %)	p-value
Age (yrs)					
Male(%)					

The following data will be summarized using the table format above:

- Demographics
- Clinical Evaluations
- Lab Tests
- Medical History
- Index PCI Results
- Index PCI Complications
- IVUS-VH Chart
- EPC Counts
- Medications Usage – Dosage

Analysis of Safety Outcomes

The primary safety endpoint and the secondary safety endpoints will be analyzed in two ways. For each type of event, the percentage of people who experience the event at least once will be compared using the chi-square test. Logistic regression models will be performed to adjust for the covariates such as age, pre-existing CAD and pre-existing diabetes. In addition, a similar analysis will be done for all AE and SAE that are possibly or probably attributable to the study intervention. An event-based analysis will also be done since adverse events can be recurrent. In this analysis, a non-parametric method called the mean cumulative function (MCF) will be used as an alternative to the above crude incidence rate analyses. The overall safety profiles as well as the safety profiles in specific subgroups will be compared for the two randomization groups including the times of event recurrence and censoring mechanisms.

Analysis of Primary Effectiveness Outcome

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

Analysis of Secondary Effectiveness Outcomes

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

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

The EPC-CFU/ml of peripheral blood assessed at 4 time points (pre-PCI, post-PCI, 30-day follow-up, and 90-day follow-up) will be analyzed via Mixed Linear models with random intercepts. The treatment effect, time effect and their interaction will be considered with or without adjusting for age, pre-existing CAD, pre-existing diabetes, baseline LDL levels, and other relevant covariates such as ACS, peripheral vascular disease, and chronic kidney disease. The statistical test of interest is the time by treatment interaction. Contrasts will be used in the Mixed Linear models to compare the differences among any specifically interested time points.

The incidence of major adverse cardiovascular endpoints (MACE) including death, myocardial infarction, coronary revascularization, and stroke will be analyzed in two ways. The percentage of people who experience the event at least once will be compared using the chi-square test. Logistic regression models will be performed to adjust for the covariates such as age, pre-existing CAD and pre-existing diabetes. An event-based analysis will also be done since MACE can be recurrent. In this analysis, a non-parametric method called the mean cumulative function (MCF) will be used as an alternative to the above crude incidence rate analyses. The overall safety profiles as well as the safety profiles in specific subgroups over the whole study period will be compared for the two randomization groups including the times of event recurrence and censoring mechanisms. Time to event (survival analysis) will also compare the time to first MACE between treatment groups. A second survival analysis will be performed to identify risk predictors and to evaluate whether the observed treatment effect is modified by adjusting for covariates such as pre-existing CAD and pre-existing diabetes.

Handling Missing Data

The Intent-to-treat (ITT) principle will be applied for primary and secondary effectiveness outcomes in which the change scores will be set to zero if the follow-up assessment is missing.

Other approaches will also be applied in order to assess the robustness of the study results for assumptions about the missing data. For outcomes about atheroma volume and % of NC component of atheroma which will be measured only at 2 time points, the sensitivity analysis approaches include: 1) performing analysis only on complete data, 2) applying the worst case scenario (set the lowest score at baseline and the highest score at 90-day follow-up), and 3) generating imputes for missing data with multiple imputation. For outcome about EPC-CFU/ml of peripheral blood which will be repeatedly measured at 4 time points, the sensitivity analysis approaches include: 1) performing analysis only on complete data, and 2) using the mixed model for repeated measures.

Plan for Presenting Data to DMC

Hines CSPCC will produce a progress report every six months for review by Data Monitoring Committee. The report will include figures for patient accrual, tables for baseline characteristics, site performance, data quality, treatment compliance, and safety issues.

The frequency, reasons and timing for withdrawal from the study will be reported and compared by treatment group. The number of misrandomized patients, i.e. ineligible patients who were randomized will be reported by treatment group. The reasons for misrandomization will be listed. The number of patients who do not receive a study intervention, receive the wrong study intervention in error or intentionally receive the study intervention to which they were not randomly assigned will be reported by treatment group. Protocol deviations will also be compared by surgical site.

	ILLT		SMT		
Withdraw	N	%	N	%	p-value
Reason ---					
Too ill to participate					
Timing ---					
Post-PCI					
30-day follow-up					

	# of Protocol Deviation			# of Patients with Deviations		
Protocol Deviation	ILLT	SMT	ALL	ILLT	SMT	ALL
Miss-randomization						
F/U outside window						

The mean group change from baseline to follow-up visits of outcome measures will be compared between the two treatment groups using a two-sample t-test:

		ILLT		SMT		Change	
Outcomes	Time	N	Mean±SD	N	Mean±SD	%Chg	p-value
Atheroma V	Baseline						
	90-day F/U						

AE and SAE will be analyzed by preferred term and system organ class. Additionally, incidence rates for AE and SAE will be analyzed by investigator attributed relatedness and severity of the events with the similar table format as the following:

	ILLT		SMT		
AE/SAE	# of Event	% of Patient	# of Event	% of Patient	p-value
AE					
SAE					

Plan for Interim Analysis

There will be no interim analysis for first stage of feasibility/pilot study due to the small sample size of 30 patients. Since DMC and FDA approve the study to continue to the second stage of pivotal study, there will be 2 interim looks, with the first look at the end of the first stage (30 patients completing the 6-month follow-up visit), and the second look will be 50% of the target sample size (about 64 patients) completing the 6-month follow-up visit as requested by FDA.

Group sequential methods will be used to specify the α-levels in order to maintain the overall significance level at $\alpha = 0.05$ for the primary effectiveness hypothesis test. The sequential analyses will use O'Brien-Fleming boundaries with an overall $\alpha = 0.05$ for significance and 90% for power. EAST software will be used to obtain the interim monitoring rule and the adjusted sample size. The first interim look will cost 0.000 for α. If the critical value calculated for the interim data is greater than 4.637 or less than -4.637, the study will be stopped with evidence that the null hypothesis H_0: $\theta = 0$ (no difference between two groups) is rejected and the ILLT group is either superior or inferior. However, if the critical value is between -4.637 and 4.637, the study will continue to the next interim look. The second interim look will cost 0.003 for α. If the critical value is greater than 2.963 or less than -2.963, the study will be stopped to reject the null hypothesis. However, if the critical value is between -2.963 and 2.963, the study will continue until the target sample size is met for final analysis.