Statistical Analysis Plan (SAP)

Section 1: Administrative

Section/Item	Index	Description
Title and trial registration	1a 1b	Assessment of prospective CYP2C19 genotype guided Dosing of Anti-Platelet Therapy in Percutaneous Coronary Intervention (ADAPT) Clinical trials.gov registration: NCT02508116
SAP Version	2	SAP Version 1.0 December 2017
Protocol version	3.	Protocol Version 3.0 date June 1, 2017
SAP revisions	4	No prior changes were made to the SAP. SAP was drafted after study completion.
SAP contributors	5	Sony Tuteja, PharmD, Co-PI Jay Giri, MD, Co-PI Henry Glick, PhD, Biostatistician
Signatures	6	Sony Tuteja Jay Giri Henry Glick

Section 2: Introduction

Section/Item	Index	Description
Background and Introduction	7	Clopidogrel is a thienopyridine antiplatelet agent, which inhibits the purinergic P2Y12 receptor on platelets and prevents their aggregation. It is commonly used in patients with acute coronary syndromes (ACS) undergoing percutaneous coronary intervention (PCI). *CYP2C19* is one of the principal enzymes involved in the bioactivation of clopidogrel from the pro-drug to its active metabolite. The most common loss of function (LOF) allele is *2 (c.681G>A; rs4244285), with frequencies of ~15% in Caucasians and Africans and 29-35% in Asians. A large meta-analysis performed by Mega and colleagues demonstrated that CYP2C19*2 carriers treated with clopidogrel have a higher risk for major adverse cardiac events compared to noncarriers (hazard ratio (HR) of 1.55, 95%CI 1.11-2.17 for heterozygotes; HR 1.76, 95% CI 1.24-2.50) and higher risk of stent thrombosis (HR 2.67, 95% CI 1.69-4.22 for heterozygotes; HR 3.97, 95% CI 1.75-9.02 for homozygotes).[1] Therefore, clopidogrel is less effective in patients who are CYP2C19 poor metabolizers and alternative therapy is recommended. While a body of observational literature and clinical guidelines[2] suggest prescribing alternative antiplatelet therapy in carriers of *CYP2C19* LOF alleles, it is unknown whether physicians will follow genotype guided recommendations when pharmacogenetic (PGx) results are presented to them in a real world setting. To provide evidence regarding the implementation and effectiveness of PGx testing following PCI, we examined the differences in prescribing of antiplatelet drugs when presented with genotype data. We also evaluated physician's adherence to the genotype guided antiplatelet recommendations.
Objectives	8	1. To determine the effect of *CYP2C19* genotyping on the prescribing patterns of antiplatelet therapies following cardiac catheterization. 2. To determine the rates of clinical outcomes. 3. To identify factors linked with successful implementation of clinical pharmacogenetic (PGx) testing in a large academic medical center.

Section 3: Study Methods

Section/Item	Index	Description
Trial Design	9	This was a prospective, pragmatic, randomized, controlled clinical pharmacogenetic implementation trial. Patients undergoing PCI with stent implantation requiring anti-platelet therapy were randomized 1:1 to either rapid genotyping of *CYP2C19* with reporting of results to the interventional cardiologist or no genotyping (usual care).
Randomization	10	We used Stata's V13.1 (College Lake, TX) random number generator to draw random numbers that were used to randomize participants to the genotyping and usual care groups. Randomization was stratified by hospital site and by the presence or absence of ACS. Acute coronary syndrome was defined as unstable angina, non-ST elevation myocardial infarction, or ST-elevation myocardial infarction. The designation of ACS vs. stable angina was made by the treating interventional cardiologist. Within each of the four groups, randomization was balanced so that after every 10 patients, 5 were randomized to the genotyped group and 5 to usual care.
Sample size	11	The sample size calculation was based on two factors: 1) the rate of pre-study prasugrel/ticagrelor use (20%) and 2) anticipated increase in prasugrel/ticagrelor prescribing based on the frequency *CYP2C19* LOF variants (~30-35%). We estimated a 15% difference in the use of prasugrel/ticagrelor in the two groups (35% in the genotyped group and 20% in the control group). A sample size of 138 per group (a total of 276) would provide 80% power at an alpha level of 0.05 to detect this difference. We increased our sample size to ~250 per group to allow for subgroup comparisons (e.g. ACS vs. stable coronary disease).
Framework	12	Genotyping will increase the prescribing of prasugrel or ticagrelor from the baseline rate of 20%.
Statistical interim analyses and stopping guidance	13	No interim analyses planned.
Timing of final analysis	14	Participants were followed until May 31, 2017 for prospective clinical endpoints assessment with the intention of a 12 month follow-up time for each participant. All analyses were performed collectively after May 31, 2017.
Timing of outcome assessment	15	Participants were called by telephone every 3 months ± 2 weeks following randomization to assess occurrence of clinical endpoints. Medical records were requested for admissions that occurred outside UPHS. Medical records within UPHS were reviewed simultaneously to assess additional clinical outcomes not reported by participants.

Section 4: Statistical Principles

Section/Item	Index	Description
Confidence intervals and p-value	16	The primary outcome was claimed at an alpha level of p=0.05.
	17	No corrections were made for multiple testing.
	18	Confidence intervals pertaining to the probability of receipt of antiplatelet agents were reported.
Adherence and protocol deviations	19a	Adherence to the genotype guided recommendations was assessed in the genotyped group by comparing the discharge antiplatelet medication to those recommended by the CPIC guidelines. The research coordinator asked the prescribing cardiologists to provide any reasons for non-adherence. Adherence was not described in the usual care group as prescribing decisions were made by the cardiologist as per usual care.
	19b	The adherence rate was defined as the number of participants in genotyped group with LOF variants that received prasugrel or ticagrelor + the number of participants without these variants that received clopidogrel divided by the total number in this group
	19c	The only protocol deviations that were encountered in this trial were enrollment of participants that did not meet the inclusion/exclusion criteria. Since this was a pragmatic clinical trial, most of the procedures were performed within the context of usual care.
	19d	Participants that did not meet inclusion/exclusion criteria and were withdrawn from the study by the treating physician or study staff.
Analysis population	20	Modified intent to treat (removing the participants that did not meet the inclusion criteria).

Section 5: Trial Population

Section/Item	Index	Description
Screening data	21	The number of PCIs performed, participants screened, and reasons for screening failure were collected by each study site. These data were reviewed by study staff to monitor recruitment and enrollment progress throughout the study.
Eligibility	22	**Inclusion Criteria** 1. Male and female subjects, ≥18 to ≤80 years at time of study 2. Status post PCI with stent implantation requiring antiplatelet therapy 3. Willingness to comply with all study-related procedures **Exclusion criteria:** 1. Pending imminent surgery placing patients at increased risk for bleeding with prasugrel or ticagrelor. 2. History of intracranial hemorrhage, TIA, and stroke 3. Active bleeding 4. Need for long-term anticoagulation (i.e. warfarin, dabigatran, rivaroxaban, apixaban, edoxaban, or lovenox). 5. Current or prior (within the past four weeks) treatment with voraxapar (Zontivity). 6. Severe renal or hepatic impairment 7. Treating physician does not want subject to participate
Recruitment	23	The number of participants assessed for eligibility along with reasons for non-enrollment will be reported as available.
Withdrawal/ follow-up	24a	The numbers of withdrawal from follow-up clinical assessments were reported as the intervention is a one-time event.
	24b	The number and time post randomization of voluntary withdrawals by participants or lost to follow-up were reported.
	24c	Reasons for voluntary withdrawal or lost to follow-up were reported if available.
Baseline patient characteristics	25a	Sex, race, age, hospital site, presence of acute coronary syndrome (ACS), insurance type, work status, tobacco use, past medical history, medications prior to admission.
	25b	For categorical variables, frequencies and percentages were reported. For continuous variables, the number of subjects, mean and standard deviation were reported by randomization group.

Section 6: Analysis

Section/Item	Index	Description
Outcome definitions	26a	**Primary outcome:** The number and proportion of participants receiving prasugrel/ticagrelor at discharge from index PCI in each randomized. Timing <7 days **Secondary Outcomes**: 1. Adherence to the CPIC guideline suggested antiplatelet therapy recommendations based on genotype (intervention group only). Timing <7 days. 2. Major adverse cardiac events (MACE) defined as cardiovascular death, myocardial infarction, stroke, urgent need for revascularization, and stent thrombosis (Academic Research Consortium "definite" stent thrombosis).[3] Timing at last follow-up. 3. Major bleeding events as defined by the Bleeding Academic Research Consortium (BARC)[4] type 3 or 5. Timing at last follow-up. 4. Non-cardiac mortality. Timing at last follow-up. 5. Implementation Metrics. Timing < 7days a. Genotype test turnaround time (Time result reported to physician – time of buccal swab). b. Number of inconclusive results.
	26b	n/a
	26c	Time to MACE or major bleed
Analysis methods	27a	**Primary outcome:** Discharge antiplatelet therapy choice by genotyped and usual care group was compared using the chi-square or Fisher's exact test. The probability of receipt of antiplatelet agents was determined by logistic regression. **Secondary outcomes:** 1. The adherence rate to the genotype guided recommendations for the genotyped group was determined by reporting the number of participants with LOF variants that received prasugrel or ticagrelor + the number of participants without these variants that received clopidogrel divided by the total number in the genotyped arm. 2. Clinical outcomes. Frequencies of CV related death, MI, stroke urgent, need for revascularization and stent thrombosis was reported as well frequencies of bleeding complications and compared using chi-square test or Fisher's exact test when appropriate. The time to first major cardiac event as a composite endpoint (CV related death, MI, stroke urgent need for revascularization and stent thrombosis) was determined. The incidence of first MACE and first major bleeding events between the groups was compared by use of Kapan-Meier estimators and statistical tests evaluated based on log-rank tests. 3. Implementation. The turnaround time for test result in the

		genotyped group was presented as mean ± standard deviation (hours).
	27b	n/a
	27c	n/a
	27d	n/a
	27e	n/a
	27f	To evaluate whether genotyping influenced MACE outcomes in participants with ACS, an interaction term between ACS and group assignment was tested in a Cox proportional hazard model.
Missing data	28	For time to MACE analysis, participants were censored at the date of event or time of known last follow-up.
Additional analyses	29	**Post-hoc analyses** Genotype was determined in the control group at the end of the trial. We combined the groups and evaluated outcomes by CYP2C19 genotype and antiplatelet drugs prescribed at discharge. Participants were grouped into the following categories: 1) Carriers of normal function alleles receiving any antiplatelet drug, 2) LOF carriers receiving clopidogrel, or 3) LOF carriers receiving prasugrel or ticagrelor. Time to MACE, major bleeding and a composite safety/efficacy endpoint consisting of MACE, major bleeding and non-cardiac mortality was determined in the three groups. Cox proportional hazards models were used to assess the effects of having an LOF allele and receiving clopidogrel therapy, having an LOF allele and receiving alternative therapy, and not having an LOF allele on the occurrence of first MACE, first major bleed, and first composite outcome. The Cox model was controlled for age at entry to the study, the date of catheterization, gender, race, hospital site, and history of ACS, smoking, hypertension, diabetes, and myocardial infarction.
Harms	30	No harms as a result of the genotyping intervention were reported.
Statistical software	31	Stata's V13.1 (College Lake, TX)
References	32	1.Mega, J.L. *et al.* Reduced-function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI: a meta-analysis. *JAMA* **304**, 1821-30 (2010). 2.Scott, S.A. *et al.* Clinical Pharmacogenetics Implementation Consortium Guidelines for CYP2C19 Genotype and Clopidogrel Therapy: 2013 Update. *Clin Pharmacol Ther* **94**, 317-23 (2013). 3.Mauri, L., Hsieh, W.H., Massaro, J.M., Ho, K.K., D'Agostino, R. & Cutlip, D.E. Stent thrombosis in randomized clinical trials of drug-eluting stents. *N Engl J Med* **356**, 1020-9 (2007). 4.Mehran, R. *et al.* Standardized Bleeding Definitions for Cardiovascular Clinical Trials: A Consensus Report From the Bleeding Academic Research Consortium. *Circulation* **123**, 2736-47 (2011).