**SAP:** TRILOGY\_Gen\_PD\_CYP2C19last updated 4.25.2013

**Project:** Impact of CYP2C19 Genetic Variants on Platelet Control and Clinical Outcomes: Insights from the TRIOLGY Pharmacogenetic Sub-Study

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**Important Deadlines:**  Abstract results by April 15, 2013; (May 1; LBCT update; ESC 2013)

**Specific Aims:**

1. Examine the relationship between CYP2C19 phenotype (EM vs. RM) on PRU response over time and examine the influence of other non-genetic clinical risk factors on this relationship.
   * Note: EM = extensive metabolizers of drug; RM = reduced metabolizers of drug
2. Examine the relationship between EM vs. RM CYP2C19 phenotype and ischemic outcomes.

**Population**

* Aim 1: All patients enrolled in TRILOGY who agreed to participate in both the genetics and platelet function sub-study, received at least one dose of study drug, and had at least one valid PRU measurement after receiving the loading or maintenance dose of the drug.
  + This is referred to as the Genetics-PD Cohort → N = 2236
* Aim 2: All patients enrolled in TRILOGY who agreed to participate in the genetics sub-study and received at least one dose of study drug.
  + This is referred to as the Genetics Cohort → N = 5736

**Endpoints:**

* Aim 1: PRU values measured at 1, 3, 6, 12, 18, 24, and 30 months post-randomization
  + Note: PRU values will be examined at 1 month and longitudinally
* Aim 2: Ischemic Outcomes
  + Composite of CV Death/MI/Stroke (primary trial endpoint)
  + CV Death
  + MI
  + Stroke
  + All-cause Death

**Analysis Objectives & Tasks:**

1. **Objective:** Describe the study cohorts and baseline patient characteristics in the overall TRILOGY cohort, in the Genetics cohort, in the Genetics-PD cohort, and then by CYP2C19 phenotype in the Genetics and Genetics-PD cohorts.

**Analysis:** Create a consort diagram describing the inclusion/exclusion criteria used to arrive at the study cohorts. Create a baseline table of patient characteristics in the overall TRILOGY cohort, the Genetics Cohort, and Genetics-PD cohort. Continuous variables will be presented as medians (Q1, Q3) and categorical variables will be presented as counts (proportions). Differences in summary measures will be tested between CYP2C19 phenotypes. Continuous variables will be compared using the ANOVA F test when the assumption of normality is satisfied; otherwise the Kruskal-Wallis test (NP) will be used. Categorical variables will be compared using the chi-square test when appropriate; otherwise an exact test (EX) will be used.

* See appendix for Figure 1: Consort Diagram of TRILOGY Genetics Substudy
* See appendix for Table 1: Baseline Characteristics in the Overall, Genetics, and Genetics-PD TRILOGY Cohorts

**Possible Conclusions:** A p-value < 0.05 would suggest that a characteristic may be imbalanced with respect to CYP2C19 phenotype. An imbalance could indicate the presence of confounding that should be taken into account when investigating the relationship between the phenotype on PRU or outcomes.

1. **Objective:** Investigate the extent of and pattern of missingness in the serial PRU values in the Genetics-PD cohort.

**Analysis:** Create spaghettis plots of PRU values across all time points overall and by CYP2C19 phenotype. Determine the median (Q1-Q3) number of PRU measurements per patient. Determine the number of patients with continuous follow-up starting at Month 1 out to each time point ending at Month 30. Determine the number of patients with breaks in their PRU measurements, when the breaks occurred, and the median (Q1 – Q3) length of the break in their trajectory.

* See appendix for Figure 2: Observed PRU Values during the Study Period
* See appendix for Table 2: Summary of PRU Measurements

**Possible Conclusions:** Understanding the pattern of missingness is important because it affects how much the missing data could bias the results as well as the robustness of the modeling approach. Once we understand the pattern of missingness, it will be important to discuss the potential mechanisms behind the missingness (e.g. lost to follow-up due to late entry in the trial or no PRU measurement because they were taken off study drug due an AE) to determine potential biases.

* Note: It will be important to look at the timing of bleeding events and how they relate the missingness to determine if the missingness is random or systematic. If the latter, the regression analyses proposed in the remainder of the SAP may need to be modified to account for these issues.

1. **Objective:** Examine the relationship between CYP2C19 phenotype and PRU values.
   1. Examine the relationship with PRU values measured at 1 month
   2. Examine the relationship with PRU values measured longitudinally

* **Clinical Hypothesis 1:** CYP2C19 phenotype will be associated with PRU values, and the association will persist across follow-up. Specifically, PRU values should be higher for RM compared to EM.
* **Clinical Hypothesis 2:** The relationship between CYP2C19 phenotype and PRU values may depend on treatment. Specifically, the difference in PRU values between RM vs. EM may be larger for clopidogrel than for prasugrel.

**Analysis:** Both clinical hypotheses will be addressed in Objective 3a and 3b.

**Model for Objective 3a**| To determine if CYP219 phenotype is associated with PRU values, a linear model with 30Day PRU value as the response and the EM vs. RM groups as a covariate. This analysis will be performed unadjusted and adjusted for race (if phenotype frequencies are found to differ by race, which is often the case), baseline PRU measurement, clopidogrel and age (above/below 75 years of age) strata, and for treatment (based on the platelet function sub-study, it is known that PRU values differ significantly between treatment groups).

**Model for Objective 3b**| To determine if CYP219 phenotype is associated with PRU values, a repeated measures analysis will be performed by fitting a mixed-effects linear model with the longitudinal PRU values as the response and the EM vs. RM groups as a covariate. The variance-covariance structure for the repeated measures will be fit with either a compound symmetry or auto-regressive (AR-1) structure. The choice of models between CS and AR-1 will be evaluated by using the AIC fit statistics, where smaller is better. This analysis will be performed unadjusted and adjusted for race (if phenotype frequencies are found to differ by race), baseline PRU value, clopidogrel and age strata, and for treatment (based on the platelet function sub-study, it is known that PRU values differ significantly between treatment groups).

* A random intercept model will be fit in this analysis. Based on the results of the platelet function sub-study, it is known that, on average, the PRU trajectories remain relatively flat across the follow-up period s starting at Month 1 (this is because steady state for prasugrel is not typically reached until 5 days after the initiation of treatment). We will recreate the series of box-and-whisker plots presented in the platelet function sub-study to determine if this holds in the Genetics-PD cohort, but there is no reason to suspect that the results would differ. As such, modeling a random slope for each subject is not warranted in this analysis. Thus, the mixed-effects model fit in this analysis will allow each subject’s expected PRU value (i.e. their intercept) to vary across the population (i.e. among subject variability) and to vary about their subject-specific trajectory across time (i.e. within subject variability). Mixed-effects models are able to handle unbalanced repeated measures data (i.e. the approach can appropriately account for unbalanced missingness when analyzing longitudinal data), but we will need to examine the patterns of missingness to determine if there are any potential sources of biases that arise.

In order to address Clinical Hypothesis 2 (under Model 3a and Model 3b), an interaction between treatment and phenotype will be included in the model and tested for significance. If a significant interaction is found, discussing the main effect of phenotype will not be appropriate; thus, any phenotype inference will be made among each treatment group. In order to address Clinical Hypothesis 1 (under Model 3a and Model 3b), the phenotype effect will be tested for significance to determine if phenotype is associated with PRU values. The relationship between phenotype and PRU values will be characterized by the EM vs. RM regression coefficient(s) and corresponding 95% confidence intervals (CIs) and p-value(s) (Note: Multiple phenotype coefficient estimates will be reported if a significant interaction is found between phenotype and treatment). Under Model 3b, a phenotype-time interaction will be tested to determine if the association of phenotype with PRU values is consistent across time.

* Objective 3a:
  + See appendix for Figure 3: 30Day PRU Values by Phenotype and Treatment
  + See appendix for Table 3: Association of Phenotype with 30 Day PRU Values
* Objective 3b:
  + See appendix for Figure 4: Typical PRU Trajectory by Phenotype and Treatment
  + See appendix for Table 4: Association of Phenotype with Longitudinal PRU Vals

**Possible Conclusions:** Under Model 3a and 3b, a significant interaction between CYP2C19 phenotype and treatment (i.e. interaction p-value < 0.05) would suggest that the effect of EM vs. RM on PRU values depends on what treatment a subject is taking. If a significant interaction is found, the phenotype effect within each treatment arm should be examined; otherwise, the phenotype effect should be examined in the entire cohort. A significant phenotype effect (i.e. phenotype p-value < 0.05) would suggest that the expected PRU value among EM vs. RM differs. Under Model 3b, a non-significant interaction between phenotype and time (i.e. phenotype-time interaction p-value ≥ 0.05) would suggest that this relationship remains constant across the study period.

1. **Objective:** Examine the relationship between CYP2C19 phenotypes and ischemic outcomes.

* **Clinical Hypothesis 1:** CYP2C19 phenotype may be associated with ischemic outcomes.
* **Clinical Hypothesis 2:** CYP2C19 phenotype may provide additional predictive information about ischemic outcomes over and above known non-genetic risk factors.

**Analysis:** To describe the relationship between CYP2C19 phenotype (EM vs. RM) and ischemic outcomes, event counts and Kaplan-Meier (KM) rates at 30 months will be calculated by CYP2C19 phenotype and by treatment. To describe timing of events, KM curves will be created for each ischemic outcome and stratified by CYP2C19 phenotype.

The relationship between CYP2C19 phenotype and ischemic outcomes will be assessed by fitting a Cox proportional hazards model for time-to-first event. The relationship between phenotype and outcome will be characterized by the EM vs. RM hazard ratio (HR) and corresponding 95% confidence interval (CI) and p-value. In order to address Clinical Hypothesis 1, a model with EM vs. RM status will be fit. This analysis will be adjusted for race (if phenotype frequencies are found to differ by race). In order to address Clinic Hypothesis 2, a model with EM vs. RM status adjusted for race (if phenotype frequencies are found to differ by race) and for known non-genetic risk factors will be fit (see the appendix for list of variables in the TRILOGY adjustment model for efficacy endpoints).

* See appendix for Table 5: Event Counts and Rates by CYP2C19 Phenotype
* See appendix for Table 6: Event Counts and Rates by Phenotype and Treatment
* See appendix for Figure 5: KM Curves for Outcome by Phenotype
* See appendix for Figure 6: KM Curves for Outcome by Phenotype and Treatment
* See appendix for Table 7: Association of CYP2C19 Phenotype with Ischemic Outcomes

**Possible Conclusions:** A p-value < 0.05 in the Cox regression modeling indicates that risk of an event differs between phenotype groups. A significant finding in Stage 1 would suggest that CYP2C19 phenotype may be causally related to clinical outcomes (after adjusting for potential race differences among phenotype groups). A significant finding in Stage 2 would suggest that CYP2C19 phenotype may provide incremental predictive information about risk over and above known clinical risk factors. It is important to note that the mechanistic pathway through which CYP2C19 phenotype affects clinical outcomes maybe through its effect on these clinical risk factors (i.e. the clinical risk factors could be intermediate factors with respect to phenotype). As such, it would be inappropriate to conclude that CYP2C19 phenotype had no relationship with outcome based on the Stage 2 analysis alone.

**Appendix A:** Covariates in the TRILOGY Adjustment Model for Efficacy Endpoints

* Study treatment (prasugrel vs. clopidogrel)
* Clopidogrel strata (stratum 3 vs. stratum 2 vs. stratum 1)
* Age (years)
* Gender (female vs. male)
* Time from randomization until study drug initiation (days?)
* Family history of CAD
* Hypertension
* Hyperlipidemia
* Diabetes
* Current or recent smoker
* Previous MI
* Previous CABG
* Previous PAD
* Previous AFib
* Previous HF
* Systolic blood pressure at baseline
* Heart rate at baseline
* Received angiography
* Hemoglobin
* Creatinine
* On beta blocker at baseline
* On ACE/ARB inhibitor at baseline
* On statin at baseline
* On PPI at baseline
* Region (7 other regions vs. Central and Eastern Europe)

**APPENDIX B:** Proposed Manuscript Tables and/or Figures

Table 1: Baseline Characteristics in the Overall, Genetics, and Genetics-PD TRILOGY Cohorts

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **TRILOGY**  **Cohort** | **Genetics**  **Cohort** | | | | **Genetics-PD**  **Cohort** | | | |
|  | **Overall** | **Overall** | **EM** | **RM** | **p-value** | **Overall** | **EM** | **RM** | **p-value** |
| (N=) | (N=) | (N=) | (N=) | (N=) | (N=) | (N=) |
| **Demographics** |  |  |  |  |  |  |  |  |  |
| Age (years) |  |  |  |  |  |  |  |  |  |
| Age < 75 yrs (%) |  |  |  |  |  |  |  |  |  |
| Female Sex (%) |  |  |  |  |  |  |  |  |  |
| Weight |  |  |  |  |  |  |  |  |  |
| Weight < 60 kg (%) |  |  |  |  |  |  |  |  |  |
| **Presentation Characteristics** |  |  |  |  |  |  |  |  |  |
| Disease Classification (%) |  |  |  |  |  |  |  |  |  |
| Unstable Angina |  |  |  |  |  |  |  |  |  |
| NSTEMI |  |  |  |  |  |  |  |  |  |
| Killip Class II-III (%) |  |  |  |  |  |  |  |  |  |
| Time from First Medical Contact to Treatment Start (hours) |  |  |  |  |  |  |  |  |  |
| **Medical History** |  |  |  |  |  |  |  |  |  |
| Family history of CAD (%) |  |  |  |  |  |  |  |  |  |
| Hypertension (%) |  |  |  |  |  |  |  |  |  |
| Hyperlipidemia (%) |  |  |  |  |  |  |  |  |  |
| Diabetes Mellitus (%) |  |  |  |  |  |  |  |  |  |
| Current/recent smoking (%) |  |  |  |  |  |  |  |  |  |
| Prior MI (%) |  |  |  |  |  |  |  |  |  |
| Prior PCI (%) |  |  |  |  |  |  |  |  |  |
| Prior CABG (%) |  |  |  |  |  |  |  |  |  |
| Prior PAD (%) |  |  |  |  |  |  |  |  |  |
| Prior Atrial Fibrillation (%) |  |  |  |  |  |  |  |  |  |
| Prior Heart Failure (%) |  |  |  |  |  |  |  |  |  |
| **Baseline Risk Assessment** |  |  |  |  |  |  |  |  |  |
| GRACE Risk Score |  |  |  |  |  |  |  |  |  |
| Creatinine Clearance (ml/min) |  |  |  |  |  |  |  |  |  |
| **Pre-Randomization Treatments** |  |  |  |  |  |  |  |  |  |
| Clopidogrel Strata (%) |  |  |  |  |  |  |  |  |  |
| Stratum 1 - no clopidogrel |  |  |  |  |  |  |  |  |  |
| Stratum 2 - clopidogrel started in-hospital<=72 hrs |  |  |  |  |  |  |  |  |  |
| Duration of Clopidogrel Use before |  |  |  |  |  |  |  |  |  |
| Treatment Start (hours) |  |  |  |  |  |  |  |  |  |
| Stratum 3 - home clopidogrel |  |  |  |  |  |  |  |  |  |
| Angiography Performed (%) |  |  |  |  |  |  |  |  |  |
| **Concomitant Medications at Randomization** |  |  |  |  |  |  |  |  |  |
| Aspirin (%) |  |  |  |  |  |  |  |  |  |
| Daily Dose < 100 mg |  |  |  |  |  |  |  |  |  |
| Daily Dose 100-250 mg |  |  |  |  |  |  |  |  |  |
| Daily Dose > 250 mg |  |  |  |  |  |  |  |  |  |
| Beta-Blocker (%) |  |  |  |  |  |  |  |  |  |
| ACE-I/ARB (%) |  |  |  |  |  |  |  |  |  |
| Statin (%) |  |  |  |  |  |  |  |  |  |
| Proton Pump Inhibitor (%) |  |  |  |  |  |  |  |  |  |

Table 2: Summary of PRU Measurements in the Genetics-PD Cohort

|  | **Overall**  **Cohort** (N=) | **Prasugrel**  **Arm** (N=) | **Clopidogrel**  **Arm** (N=) |
| --- | --- | --- | --- |
| **Number of time points per patient *Median (Q1, Q3)*** |  |  |  |
|  |  |  |  |
| **Patient has a 30-day result** |  |  |  |
|  |  |  |  |
| **Patients with continuous follow-up after 30-day time point** |  |  |  |
| **Patients with complete follow-up after 30-day** |  |  |  |
| **Patients with incomplete follow-up after 30-day but no gaps** |  |  |  |
| **Last time point available** |  |  |  |
| **Month 1** |  |  |  |
| **Month 3** |  |  |  |
| **Month 6** |  |  |  |
| **Month 12** |  |  |  |
| **Month 18** |  |  |  |
| **Month 24** |  |  |  |
| **Month 30** |  |  |  |
|  |  |  |  |
| **Patients without continuous follow-up after 30-day time point** |  |  |  |
| **Length of break (days) *Median (Q1, Q3)*** |  |  |  |
| **When break occurred** |  |  |  |
| **Month 3** |  |  |  |
| **Month 6** |  |  |  |
| **Month 12** |  |  |  |
| **Month 18** |  |  |  |
| **Month 24** |  |  |  |

Table 3: Association of CYP2C19 Phenotype with 30 Day PRU Values

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Stage 1** (Unadjusted) | | | **Stage 2** (Adjusted) ‡ | | |
|  | Estimate | 95% CI | Intx p-value† | Estimate | 95% CI | Intx p-value† |
| **EM vs. RM Difference**‡ |  |  |  |  |  |  |
| Prasugrel Arm |  |  |  |  |  |  |
| Clopidogrel Arm |  |  |  |  |  |  |
| † P-value for the interaction between CYP2C19 Phenotype (EM vs. RM) and Treatment (prasugrel vs. clopidogrel)  ‡ Adjusted for clopidogrel strata, age strata (above/below 75 years), baseline PRU value, and treatment. | | | | | | |

Table 4: Association of CYP2C19 Phenotype with Longitudinal PRU Values

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Stage 1** (Unadjusted) | | | **Stage 2** (Adjusted) ‡ | | |
|  | Estimate | 95% CI | Intx p-value† | Estimate | 95% CI | Intx p-value† |
| **EM vs. RM Difference**‡ |  |  |  |  |  |  |
| Prasugrel Arm |  |  |  |  |  |  |
| Clopidogrel Arm |  |  |  |  |  |  |
| † P-value for the interaction between CYP2C19 Phenotype (EM vs. RM) and Treatment (prasugrel vs. clopidogrel)  ‡ Adjusted for clopidogrel strata, age strata (above/below 75 years), baseline PRU value, and treatment. | | | | | | |

Table 5: Event Counts and Rates by CYP2C19 Phenotype

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **EM Phenotype** | | **RM Phenotype** | |  |
|  | Event Count | KM Rate  at 30 Months | Event Count | KM Rate  at 30 Months | p-value |
| **CV Death/MI/Stroke** |  |  |  |  |  |
| **CV Death** |  |  |  |  |  |
| **MI** |  |  |  |  |  |
| **Stroke** |  |  |  |  |  |
| **All-Cause Death** |  |  |  |  |  |

Table 6: Event Counts and Rates by CYP2C19 Phenotype and Treatment

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **EM Phenotype** | | | | **RM Phenotype** | | | |  |
|  | **Clopidogrel** | | **Prasugrel** | | **Clopidogrel** | | **Prasugrel** | |  |
|  | Event Count | KM Rate  at 30 Mths | Event Count | KM Rate  at 30 Mths | Event Count | KM Rate  at 30 Mths | Event Count | KM Rate  at 30 Mths | p-value |
| **CV Death/MI/Stroke** |  |  |  |  |  |  |  |  |  |
| **CV Death** |  |  |  |  |  |  |  |  |  |
| **MI** |  |  |  |  |  |  |  |  |  |
| **Stroke** |  |  |  |  |  |  |  |  |  |
| **All-Cause Death** |  |  |  |  |  |  |  |  |  |

Table 7: Association of CYP2C19 Phenotype (EM vs. RM) with Ischemic Outcomes

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Stage 1** (Unadjusted) | | | | **Stage 2** (Adjusted) ‡ | | | |
|  | EM vs. RM HR | 95% CI | p-value | Intx p-value† | EM vs. RM HR | 95% CI | p-value | Intx p-value† |
| **CV Death/MI/Stroke** |  |  |  |  |  |  |  |  |
| **CV Death** |  |  |  |  |  |  |  |  |
| **MI** |  |  |  |  |  |  |  |  |
| **Stroke** |  |  |  |  |  |  |  |  |
| **All-Cause Death** |  |  |  |  |  |  |  |  |
| † P-value for the interaction between CYP2C19 Phenotype (EM vs. RM) and Treatment (prasugrel vs. clopidogrel).  ‡ Adjusted for all variables in the TRILOGY efficacy adjustment models; for full list see Appendix A | | | | | | | | |

Figure 1: Consort Diagram of the TRILOGY Genetics Substudy

Figure 2: PRU Values during the Study Period in the Genetics-PD Cohort

Figure 3: 30Day PRU Values by CYP219 Phenotype and Treatment in the Genetics-PD Cohort

Figure 4: Typical PRU Trajectory by CYP2C1 Phenotype and Treatment

Figure 5: Kaplan-Meier Curves for Ischemic Outcome by CYP2C19 Phenotype

* Figure 5a: Kaplan-Meier Curves for CV Death/MI/Stroke by CYP2C19 Phenotype
* Figure 5b: Kaplan-Meier Curves for CV Death by CYP2C19 Phenotype
* Figure 5c: Kaplan-Meier Curves for MI by CYP2C19 Phenotype
* Figure 5d: Kaplan-Meier Curves for Stroke by CYP2C19 Phenotype
* Figure 5e: Kaplan-Meier Curves for All-Cause Death by CYP2C19 Phenotype

Figure 6: Kaplan-Meier Curves for Ischemic Outcome by CYP2C19 Phenotype and Treatment

* Figure 5a: Kaplan-Meier Curves for CV Death/MI/Stroke by CYP2C19 Phenotype and Treatment
* Figure 5b: Kaplan-Meier Curves for CV Death by CYP2C19 Phenotype and Treatment
* Figure 5c: Kaplan-Meier Curves for MI by CYP2C19 Phenotype and Treatment
* Figure 5d: Kaplan-Meier Curves for Stroke by CYP2C19 Phenotype and Treatment
* Figure 5e: Kaplan-Meier Curves for All-Cause Death by CYP2C19 Phenotype and Treatment