# A Pharmacodynamic study comparing prasugrel versus ticagrelor in patients with coronary artery disease undergoing PCI with CYP2C19 loss-of-function genotypes: A feasibility study with point-of-care pharmacodynamic and genetic testing

**Investigators**

Dominick J Angiolillo, MD, PhD

Fabiana Rollini, MD

Francesco Franchi, MD

Jennifer Dover, RN, BSN

**Project Overview**

Clopidogrel is the most broadly utilized platelet $P2Y_{12}$ receptor inhibitor. However, numerous studies have shown that pharmacodynamics (PD) response profiles vary among clopidogrel treated patients and that individuals with reduced response have an increased risk of recurrent ischemic events. There are multiple factors contributing to clopidogrel response variability. Among these, genetic variations of the cytochrome P450 (CYP) 2C19 enzyme, a key contributor to clopidogrel metabolism, have been involved. In particular, loss-of-function (LOF) alleles of the CYP2C19 enzyme reduce transformation of clopidogrel pro-drug into its active metabolite. Thus, patients carrying LOF alleles have lower levels of clopidogrel's active metabolite as well as diminished platelet inhibition, which translates into an increased rate of adverse cardiovascular events, particularly in the setting of percutaneous coronary intervention (PCI). Because of these findings, drug regulating authorities have provided a boxed warning on the product label of clopidogrel on the potential for reduced efficacy of clopidogrel among CYP2C19 LOF carriers and suggested considering alternative antiplatelet therapies for these individuals. Prasugrel and ticagrelor are novel generation $P2Y_{12}$ receptor inhibitors characterized by greater PD potency and reduced ischemic event rates compared with clopidogrel, and are not affected by CYP2C19 LOF polymorphisms. However, to date there are limited head-to-head PD comparisons between these two new $P2Y_{12}$ receptors blockers, and there are no studies assessing on how these agents behave among CYP2C19 LOF carriers. Tailoring antiplatelet therapy according to results of genetic testing has been limited in real world clinical practice because of not having readily accessible results of individual's genetic makeup. The aim of the present study is to compare the PD effects of prasugrel versus ticagrelor in patients undergoing PCI with CYP2C19 LOF alleles using the novel point-of-care genetic testing *Spartan RX-CYP2C19* which permits accurate and rapid identification of CYP2C19 genetic status.

**Background and Significance:**

Dual antiplatelet therapy consisting of aspirin and clopidogrel is the cornerstone of treatment for prevention of thrombotic events in patients with coronary artery disease (CAD) who have an acute coronary syndrome (ACS) or undergo percutaneous coronary intervention (PCI) [1]. However, there are a considerable number of patients who continue to have recurrent ischemic events despite this treatment regimen [2,3]. In fact, variability in the pharmacodynamic (PD) response to clopidogrel is well recognized, and patients with high on-treatment platelet reactivity (HPR) are at increased risk of adverse cardiovascular events [4]. Variability in clopidogrel response is a multifactorial process. One of the contributing factors is the variability in its metabolism. Clopidogrel is a prodrug and needs to undergo biotransformation to become an active metabolite to exert its antiplatelet effects. Clopidogrel is metabolized by the hepatic cytochrome P450 (CYP) system in two steps and CYP 2C19 is involved in both steps [5]. Multiples studies have demonstrated that both heterozygotes and homozygotes for loss-of-function (LOF) alleles have lower levels of the active clopidogrel metabolite as well as diminished platelet inhibition [6]. Moreover, carriers of any LOF allele have an increased rate of cardiovascular adverse events, particularly in the setting of PCI [7]. Because of these findings, drug regulating authorities have provided a boxed warning on the product label of clopidogrel on the potential for reduced efficacy of clopidogrel among CYP2C19 LOF carriers and suggested considering alternative antiplatelet therapies, which include prasugrel and ticagrelor, for these individuals [8,9]. However, to date there are no head-to-head comparisons between these agents among CYP2C19 LOF carriers.

Recently, new $P2Y_{12}$ receptor blockers, prasugrel and ticagrelor, have been approved for clinical use [10,11]. Both new $P2Y_{12}$ receptor antagonists have more potent PD effects compared with clopidogrel, which has translated into better clinical outcomes in patients with ACS [12,13]. The PD

effects of both drugs have not shown to be affected by CYP2C19 genetic status and both agents achieve more potent platelet inhibitory effects than clopidogrel in healthy volunteers with CYP2C19 LOF alleles [6,14]. These PD findings have also been shown in patients with CAD and post-hoc analyses of two large, international trials have not shown any clinical interaction between ticagrelor or prasugrel and CYP2C19 LOF polymorphisms [15-20]. However, to date there are no head-to-head PD comparisons between these two new $P2Y_{12}$ receptors blockers among patients with CYP2C19 LOF alleles. Tailoring antiplatelet therapy according to results of genetic testing has been limited in real world clinical practice of patients undergoing PCI by having readily accessible results of individual's genetic makeup. Recently, a new point-of-care genotyping assay for the CYP2C19 LOF polymorphisms, *Spartan RX*, has been developed and approved by the Food and Drug Administration (FDA) as a feasible bedside tool [21,22]. The Rapid Gene study showed that point-of-care genetic testing permits accurate identification of CYP2C19 LOF carriers. Moreover, the use of Spartan RX as genetic testing may facilitate the rapid personalization of anti-platelet therapy as demonstrated with the reduction of HPR in the rapid genotyping group using prasugrel on those patients who showed to be LOF carriers [18,20]. The present investigation will compare the PD effects of prasugrel and ticagrelor in PCI-treated patients with CYP2C19 LOF using the *Spartan RX* device.

**Specific Aim and study hypothesis:**

The primary aim of the present study is to compare the PD effects of prasugrel and ticagrelor in patients undergoing PCI presenting with CYP2C19 LOF alleles. The study hypothesis is the non-inferiority in platelet reactivity at 24 hours of prasugrel versus ticagrelor among LOF allele carriers.

**Research Design and Methods:**

***Research design***

Patients scheduled for left heart catheterization (LHC) with intent to undergo PCI will be screened for the study. Patients will be screened in our inpatient and outpatients services referring patients to our cardiac catheterization laboratories to undergo LHC. Patients must fulfill all specific inclusion and exclusion criteria described below. A rapid genetic testing using the point-of-care genotyping (*Spartan RX*) will be performed in these patients to define carriers of LOF alleles. In particular, this point-of-care assay enables assessment of the following alleles: *1,*2,*3 and *17. The most common LOF alleles are *2 and *3. Therefore, carriers of *2 or *3 LOF carrier status [homozygotes (*2/*2, *3/*3 or *2/*3) or heterozygotes (*1/*2, *1/*3, *2/*17, *3/*17)] will be eligible for randomization. Patients who are non-carriers of LOF alleles (*1/*1, *1/*17 or *17/*17) will not be eligible for randomization and considered as screen failures and treated per standard of care. Also, screened patients with CYP2C19 LOF alleles who do not undergo PCI will be considered as screen failures and treated per standard of care.

Screened patients, either on aspirin monotherapy or on dual antiplatelet therapy (DAPT) with aspirin and clopidogrel as per standard of care, defined to be carriers of LOF and undergoing PCI will be randomized. Patients will be randomly (1:1 randomization) assigned to receive FDA approved doses of either prasugrel (60 mg loading dose - 10 mg/day maintenance dose) or ticagrelor (180 mg loading dose - 90 mg b.i.d maintenance dose). Randomization will be stratified according to baseline antiplatelet therapy (aspirin alone vs. DAPT with aspirin and clopidogrel). Loading dose administration will be given immediately after PCI as per standard of care. Maintenance dose will be

maintained for up to 30 days. Afterwards, patients will continue with the antiplatelet therapy recommended by the treating cardiologist (clopidogrel, prasugrel or ticagrelor).

***Study Population***

• ***Inclusion criteria:***

1. Patients scheduled for left heart catheterization and undergoing PCI
2. Age 18-75 years
3. On aspirin (81mg) monotherapy or on DAPT with aspirin (81mg) and clopidogrel (75mg) as per standard of care
4. Presence of at least one 2C19 LOF allele

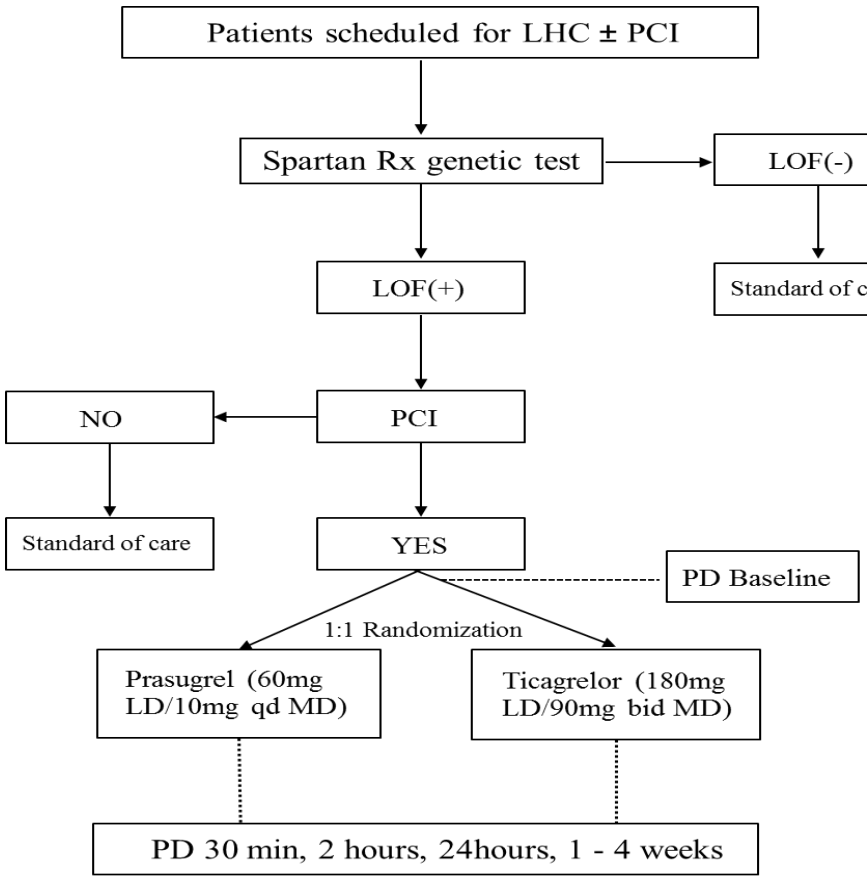
• ***Exclusion criteria:***

1. Known allergies to aspirin, prasugrel, ticagrelor, or clopidogrel
2. Age >75 years
3. Weight <60kg
4. Considered at high risk for bleeding
5. History of ischemic or hemorrhagic stroke or transient ischemic attack
6. Known severe hepatic dysfunction
7. On treatment with oral anticoagulant therapy (Vitamin K antagonists, dabigatran, apixaban, rivaroxaban)
8. Use of glycoprotein IIb/IIIa inhibitors (abciximab, eptifibatide, tirofiban)
9. Blood dyscrasia or bleeding diathesis
10. Platelet count $<80x10^6$/mL

11. Hemoglobin <10 g/dL.
12. Active bleeding or hemodynamic instability
13. Creatinine Clearance <30 mL/minute
14. Patients with sick sinus syndrome (SSS) or high degree AV block without pacemaker protection.
15. Current treatment with drugs interfering with CYP3A4 metabolism (to avoid interaction with Ticagrelor): Ketoconazole, itraconazole, voriconazole, clarithromycin, nefazodone, ritonavir, saquinavir, nelfinavir, indinavir, atazanavir, and telithromycin.
16. Pregnant females*

*Women of childbearing age must use reliable birth control (i.e. oral contraceptives) while participating in the study.

The study design is detailed in the following figure.



***Blood sampling***

In patients meeting study entry criteria and undergoing PCI, blood sampling will be performed at 5 time points: a) baseline (prior to initiating the PCI procedure and loading dose administration of antiplatelet therapy); b) 30 minutes after loading dose administration; c) 2 hours after loading dose administration; d) 24 hours after loading dose administration or hospital discharge (whichever comes first); e) during routine follow-up clinical visit 1 – 4 weeks after PCI while on maintenance dose antiplatelet therapy. Time points will have a 10% window.

***Genetic Testing***

*Spartan RX point-of-care rapid genotyping*: Spartan RX (Spartan Bioscience Inc., Ontario, Canada) is the point-of-care determining the CYP2C19 (*1,*2,*3,*17) allele status within 1 hour. This test consisted of four separate steps intended to be done in less than 8 minutes: acquisition of a buccal swab; insertion of the swab into the cartridge; insertion of the reaction solution into the device; and analysis of CYP2C19 genotype triggered by a button on the device. In this study, patients with the *2 or *3 LOF carrier status [homozygotes (*2/*2, *3/*3 or *2/*3) or heterozygotes (*1/*2, *1/*3,

*2/*17, *3/*17)] will be eligible for randomization. Patients who are non-carriers of LOF alleles (*1/*1, *1/*17 or *17/*17) will be treated per standard of care [21].

***Platelet Function Assays:***

*VerifyNow Point-of-care Testing*: The VerifyNow System is a turbidimetric based optical detection system which measures platelet induced aggregation as an increase in light transmittance (Accumetrics, San Diego, CA) and will be utilized according to manufacturer's instructions [23]. The assay is based on microbead agglutination and uses specific reagents for the pathways of interest. The instrument measures this change in optical signal and reports results in $P2Y_{12}$ Reaction Units (PRU). High on-treatment platelet reactivity (HPR) is defined by PRU >208 [4].

***Study Endpoints and Determination of Sample Size:***

The primary endpoint is the non-inferiority in platelet reactivity, measured as PRU, at 24 hours or hospital discharge (whichever comes first) of prasugrel versus ticagrelor among LOF allele carriers in each cohort. Under the assumption of 0 difference in mean PRU between treatments and a common standard deviation of 50 PRU, a sample size of 60 patients will allow for the 95% CI to stay within ± 40 PRU with a 85% power and alpha=0.025. Considering a 25% rate of invalid results due to hemolysis or drop-out (based on our prior experience), we will randomize a total of 80 patients to ensure complete data for analysis. Non-inferiority will be assessed using a 95% CI of the difference in mean PRU between the 2 groups. Secondary endpoints will include: differences in PRU between prasugrel and ticagrelor at 30 minutes, 2 hours, and 1-4 weeks (during maintenance therapy); rates of HPR at all study time points. Subgroup analyses will be performed according to baseline antiplatelet therapy (aspirin alone vs. DAPT with aspirin and clopidogrel)

***Statistical Analysis:***

Continuous variables will be expressed as a mean ± SD or median [IQR] as appropriate. Categorical variables will be expressed as frequencies and percentages. Comparisons between continuous variables will be performed using Student t-test or Wilcoxon rank-sum test. Comparisons between categorical variables will be performed using McNemar test or binomial exact test. Missing data will not be imputed. An analysis of covariance method with a general linear model, with treatment as the main effect and baseline values of platelet reactivity as a covariate, will used to evaluate the primary endpoint as well as all between-group comparisons at each time point. Least squares mean (LSM) differences in PRU between groups and the corresponding 2-sided 95% CI for the difference will be obtained based on the analysis of covariance model. The PD population will include all patients with PD data and without a major protocol deviation thought to affect significantly the PD effects of ticagrelor or prasugrel. The PD population will be used for analysis of all primary and secondary PD variables. Erroneously treated patients (e.g., those randomized to one treatment but actually given the other) will be accounted for based on the actual treatment received. Statistical analysis will be performed by our group using SPSSv24.0 software (SPSS Inc. Chicago, IL).

**Possible Discomforts and Risk**

In clinical trials, the most common clinical side effects of prasugrel were blurred vision, dizziness, headache, nervousness; infrequent events included intracranial hemorrhage (0.79%) and severe neutropenia (< 0.1%). The most important adverse effect associated with the use of prasugrel is bleeding [11]. The risk of non-surgical bleeding is 2.4%. On the other hand, the most common clinically side effects of ticagrelor were dyspnea (13.8%), headache (6.5%), cough (4.9%), dizziness

(4.5%), and nausea (4.3%), principally. Also, the most important adverse effect associated with the use of ticagrelor is bleeding [10]. The risk of non-surgical bleeding is 2.8%. All clinical events, if they were to occur, including myocardial infarctions both fatal and non-fatal, strokes, peripheral vascular disease, bypass surgery (coronary or peripheral vascular), repeated hospitalizations, and bleeding will be recorded. Clinical events will be evaluated by a local committee, comprised of 3 faculty members (2 cardiologists and 1 non-cardiologist), not directly involved in the research. In the event of a report of a serious adverse event (major bleeding – defined as life-threatening: fatal, symptomatic intracranial hemorrhage, leading to a drop in hemoglobin of at least 5 g/dL, significant hypotension requiring intravenous inotropes, requiring surgical intervention, or requiring transfusion of 4 or more units of blood; non–life-threatening: substantially disabling, intraocular bleeding leading to vision loss, or requiring at least 2 units of blood; thrombocytopenia <50,000 ), the local committee will meet and antiplatelet treatment will be withdrawn.

**Definition of Adverse Events**

An adverse event is any unintended or undesirable experience that occurs during the course of the clinical investigation whether or not it is considered to be therapy related. This includes any newly occurring event or previous condition that has increased in severity or frequency since the initiation of study treatment. Adverse events will be followed until resolution while the patient remains on-study. Once the patient is removed from study, events thought to be related to the study therapy will be followed until resolution or until the patient starts a new treatment regimen.

Serious Adverse Events (SAE): An adverse event occurring while on study and considered related (reasonable possibility that the study treatment caused the adverse experience) to the study treatment that results in any of the following outcomes:

- Death

- A life-threatening adverse experience.

- A persistent or significant disability, incapacity, or is a congenital anomaly, or birth defect.

- Requires inpatient hospitalization, or prolongation of existing hospitalization.

The definition of serious adverse event also includes 'important medical event'. Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient and/or may require medical or surgical intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

**Possible benefits**

The present investigation is aimed to evaluate the PD differences between ticagrelor and prasugrel in patients with CYP2C19 LOF alleles. Although, this study is not designed to evaluate differences in clinical outcomes, the results obtained may prompt further large scale clinical investigation.

**Potential Financial Risks**

None

**Potential Financial Benefits**

None

**Conflict of Interest**

Dr. Angiolillo is a consultant for Bristol Myers Squibb/Sanofi-Aventis, the makers of clopidogrel (Plavix), Eli-Lilly/Daiichi-Sanyo, the makers of prasugrel (Effient), and Astra Zeneca, the makers of ticagrelor (Brilinta).

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