Pharmacodynamic Effect of Switching Therapy in Patients With High On-Treatment Platelet Reactivity and Genotype Variation With High Clopidogrel Dose Versus Prasugrel

The RESET GENE Trial

Gennaro Sardella, MD, FACC, FESC; Simone Calcagno, MD; Massimo Mancone, MD, PhD; Raffaele Palmirotta, MD; Luigi Lucisano, MD; Emanuele Canali, MD; Rocco Edoardo Stio, MD; Mauro Pennacchi, MD; Angelo Di Roma, MD; Giulia Benedetti, MD; Fiorella Guadagni, MD; Giuseppe Biondi-Zoccai, MD; Francesco Fedele, MD

**Background**—High on-treatment platelet reactivity (HTPR) is associated with adverse outcomes. We aim to compare the novel thienopyridine prasugrel versus double-dose clopidogrel in patients with HTPR and explore the interaction between CYP2C19 genotype and both drugs.

**Methods and Results**—Consecutive stable patients undergoing percutaneous coronary intervention were screened with the Multiplate Analyzer P2Y12 assay, defining HTPR as area under the curve >450. Those with HTPR were randomized to prasugrel (10 mg/day) or high-dose clopidogrel (150 mg/day) for 2 weeks and then crossed-over to, respectively, clopidogrel and prasugrel, repeating the P2Y12 assay at the end of each cycle. Clinical follow-up (until 3 months) and CYP2C19 genotyping was performed in all patients. The primary end point was platelet reactivity after 14 days of prasugrel versus high-dose clopidogrel. Thirty-two patients were randomized to prasugrel and then high-dose clopidogrel or to high-dose clopidogrel followed by prasugrel. Prasugrel was associated with a significantly lower platelet reactivity than high-dose clopidogrel was (325.8 versus 478.5 area under the curve, \( P=0.028 \)). No patient treated with prasugrel exhibited HTPR, whereas 9 (28.1%) receiving high-dose clopidogrel still had prevalence of HTPR (\( P=0.001 \)). Similar findings were obtained changing cutoffs or considering platelet reactivity as a continuous variable. Genotyping showed the same efficacy between high-dose clopidogrel and prasugrel in the 18 (56.3%) CYP2C19*2 noncarriers (HTPR in 12.5% versus 0, \( P=0.274 \)), whereas it was significantly worse in the 14 (43.7%) carriers (HTPR in 43.7% versus 0, \( P=0.003 \)).

**Conclusions**—HTPR is successfully abolished by therapy with prasugrel irrespective of CYP2C19 genotype. Conversely, high-dose clopidogrel can address HTPR only in CYP2C19*2 noncarriers.

**Clinical Trial Registration**—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01465828. (Circ Cardiovasc Interv. 2012;5:698-704.)

**Key Words:** clopidogrel ■ coronary artery disease ■ platelet reactivity ■ prasugrel

Dual antiplatelet therapy with aspirin and clopidogrel has become the cornerstone preventive means of ischemic events in patients undergoing percutaneous coronary intervention (PCI). However, substantial between-subject variability in platelet response (PR) to clopidogrel has been reported, with several mechanisms being implicated for high on-treatment platelet reactivity (HTPR). The mechanisms leading to a poor response to clopidogrel are not clearly understood; clinical, cellular, and genetic factors have been proposed.

Previous investigations have shown that administration of a higher clopidogrel loading dose enhances platelet inhibition and leads to better response profiles than standard dosing does. Increasing the usual 75 mg/day clopidogrel to 150 mg/day seems to be a reasonable approach to overcome low responsiveness.

The P2Y12 inhibition achieved by prasugrel, because of metabolic differences in the generation of the active metabolite, occurs more rapidly, consistently, and to a greater extent than with standard clopidogrel. In vitro and in vivo studies
of prasugrel indicate minor contribution of CYP2C9 and CYP2C19 to its metabolic activation,15 but optimization of post-PCI platelet inhibition in patients with HTPR remains a controversial issue, with little information available about the use of strategies of more benefit for such patients.

The primary aim of the present study was to investigate the antiplatelet effects of prasugrel (10 mg/day) versus high-dose clopidogrel (150 mg/day) in stable patients with HTPR, also taking into account genotype variation.

**WHAT IS KNOWN**

- Substantial patient variability in platelet response to clopidogrel has been reported with high on-treatment platelet reactivity in a significant proportion of patients.
- Treatment with prasugrel is associated with greater platelet inhibition than treatment with high-dose clopidogrel.
- Loss-of-function alleles in CYP2C19 results in a reduced availability of clopidogrel with a consequently lower antiplatelet effect.

**WHAT THE STUDY ADDS**

- This study confirmed that prasugrel is associated with greater platelet inhibition than high-dose clopidogrel, and this effect is seen more in CYP2C19*2 carriers.
- CYP2C19*2 carriers could be identified by an area under the curve cutoff value (600 area under the curve).

### Methods

**Design**

The pharmacodynamic effects of switching theRapy in patiEntS with CYP2C19 to its metabolic activation,15 but optimization of post-PCI platelet inhibition in patients with HTPR remains a controversial issue, with little information available about the use of strategies of more benefit for such patients.

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according to the Academic Research Consortium statement, target vessel revascularization or stroke), major bleedings (Bleeding Academic Consortium 3–5), minor bleeding (Bleeding Academic Consortium 2), and (net adverse clinical events, ie, the composite of major adverse cerebrovascular events and major bleedings) up to 12 months.

**Statistical Analysis**

The sample size was computed hypothesizing that prasugrel therapy would result in a platelet function absolute difference of 150 AUC (35% relative reduction) compared with high-dose clopidogrel (with the assumption that the within-patient SD of the response variable is 120 AUC) based on previously published data. Choosing a 95% power and a 2-sided 5% \( \alpha \), at least 32 patients (16 for each group) were needed. Normal distribution was tested with the Kolmogorov–Smirnov test. Continuous variables are reported as means\( \pm \)SD, and categorical ones as n (%). Pearson \( \chi^2 \) test was used for comparison of categorical data, whereas a Student \( t \) test for independent groups was used to assess differences in continuous variables. In addition, period and sequence effects, which may occur in crossover studies, were evaluated to perform an unbiased estimation of the treatment effect. To test for sequence treatment effects, we evaluated the functional end points within the 2 treatment sequences in which patients received either prasugrel or clopidogrel. In particular, we compared the precrossover with the postcrossover functional values within the treatment sequence in which patients were randomized to clopidogrel first and then prasugrel, and within the other sequence in which patients were randomized to prasugrel first and then clopidogrel. Then, the absolute mean differences between the precrossover and the postcrossover values and comparisons of these between-treatment differences achieved in each sequence were computed. To test for period effects, for each sequence the average of the difference of the 2 periods and the sum of these 2 averages were calculated and then the 2 achieved averages of each sequence were compared. Only patients who successfully completed \( \geq 1 \) period of the study were considered for analysis. Receiver-operating characteristic curves were used to identify the best cutoff value of baseline platelet function to detect subjects with the loss-of-function allele CYP2C19*2. Statistical significance was set at the 2-sided 0.05 level. Computations were performed with SPSS 17 (IBM, Armonk, NY).

**Results**

Of 180 patients with PR assessment, 42 patients (23%) with HTPR were identified, and 32 patients were finally enrolled and randomly assigned to either prasugrel followed by high-dose clopidogrel or high-dose clopidogrel followed by prasugrel (Figure 1), as 10 patients were excluded from randomization because of absolute contraindications to prasugrel assumption. All enrolled subjects completed both treatment periods, thus serving as their own control and were evaluated for genotype assessment. Patient baseline characteristics are described in Table 1.

At day 0, pharmacodynamic analyses showed (Table 2) no significant difference in PR in the 2 groups (576±97.2 AUC in the prasugrel group and 573.3±87.1 AUC in the high-dose clopidogrel group, \( P=0.957 \)).

After treatment with prasugrel, patients had a significantly greater inhibition of platelet aggregation compared with treatment with high-dose clopidogrel (49.7±42.9% versus 9.3±5.2%, \( P=0.036 \)). In the precrossover period, prasugrel decreased AUC more than high-dose clopidogrel did (180.5±40.6 versus 380.5±60.1 respectively, \( P<0.001 \)). After crossover patients initially allocated to the clopidogrel group presented a remarkable lower AUC (Figure 2), in contrast with the other group, which showed an increased AUC after switching to clopidogrel (330±100.8 versus 256±157.2, respectively, \( P=0.07 \)).

Analysis of individual response according to treatment showed that in some patients values of AUC >450 persisted after treatment with clopidogrel (Figure 3), whereas no such case occurred after prasugrel treatment (9 [28.1 versus 0%], respectively; \( P=0.001 \)). Genotyping revealed at least carriage of 1 CYP2C19*2 loss-of-function allele in 14 patients (43.7%) with 2 (12.5%) homozygotes identified. PR was significantly lower (\( P=0.045 \)) for prasugrel in carriers (Figure 4), whereas no differences were observed in noncarriers (\( P=0.575 \)). Although our study was not designed and adequately powered for this analysis, 6 (43.7%) of 14
carriers continued to demonstrate HTPR despite high-dose clopidogrel, whereas no one remained a poor responder to prasugrel ($P=0.003$). Two (12.5%) of 18 noncarriers remained poor responders to clopidogrel, whereas all responded to prasugrel ($P=0.274$). The prevalence of HTPR (ie, AUC >450) in noncarriers and carriers, separately for each treatment arm, did not differ significantly (43.7 versus 12.5%, $P=0.248$, for clopidogrel and 0 in both groups, $P=1.0$, for prasugrel, respectively).

During the study period 7 patients, initially allocated to prasugrel, had ≥1 adverse event: 3 were minor bleedings, and 4 were uncomplicated chest pain events. In patients allocated to clopidogrel 3 had an event: 1, minor bleeding, and 2, uncomplicated chest pain event.

Analysis of the receiver-operating characteristic curve identified a 600 AUC cutoff for the identification of carriers of the CYP2C19*2 allele, with 75% sensibility and 72% specificity (95% CI; $P=0.032$; Figure 5).

**Discussion**

The RESET GENE trial confirms the hypothesis that treatment with prasugrel is associated with greater platelet inhibition than treatment with high-dose clopidogrel in a prospective study enrolling stable patients after PCI. After therapy with prasugrel there were no nonresponders, whereas several patients remained nonresponders despite treatment with high-dose clopidogrel.

Similar findings to those presently reported have been found in other studies, where patients treated with prasugrel had significantly lower rates of HTPR compared with patients treated with clopidogrel. Specifically, the clinical impact of

### Table 1. Patient Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Patients Randomized to Prasugrel Followed by High-Dose Clopidogrel (n=16)</th>
<th>Patients Randomized to High-Dose Clopidogrel Followed by Prasugrel (n=16)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>14 (87.5%)</td>
<td>13 (83.3%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Age, y</td>
<td>61.8±10.4</td>
<td>62.2±8.6</td>
<td>0.90</td>
</tr>
<tr>
<td>Bodymass index, kg/m²</td>
<td>27.8±3.6</td>
<td>28.3±2.7</td>
<td>0.65</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td>0.98±0.61</td>
<td>0.86±0.30</td>
<td>0.48</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>10 (62.5%)</td>
<td>8 (50.0%)</td>
<td>0.47</td>
</tr>
<tr>
<td>Hypertension</td>
<td>12 (75.0%)</td>
<td>11 (68.7%)</td>
<td>0.69</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4 (25.0%)</td>
<td>5 (31.2%)</td>
<td>0.69</td>
</tr>
<tr>
<td>Smoking</td>
<td>8 (50.0%)</td>
<td>8 (50.0%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Prior myocardial infarction</td>
<td>6 (37.5%)</td>
<td>3 (18.7%)</td>
<td>0.23</td>
</tr>
<tr>
<td>Prior percutaneous coronary intervention</td>
<td>6 (37.5%)</td>
<td>3 (18.7%)</td>
<td>0.23</td>
</tr>
<tr>
<td>Prior coronary artery bypass grafting</td>
<td>1 (6.3%)</td>
<td>0</td>
<td>0.30</td>
</tr>
<tr>
<td>Medical therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>12 (75.0%)</td>
<td>14 (87.5%)</td>
<td>0.36</td>
</tr>
<tr>
<td>Proton pump inhibitors</td>
<td>9 (56.2%)</td>
<td>10 (62.5%)</td>
<td>0.71</td>
</tr>
<tr>
<td>β-blockers</td>
<td>10 (62.5%)</td>
<td>10 (62.5%)</td>
<td>1</td>
</tr>
<tr>
<td>Nitrates</td>
<td>4 (25.0%)</td>
<td>6 (37.5%)</td>
<td>0.44</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme-inhibitors</td>
<td>6 (37.5%)</td>
<td>4 (25.0%)</td>
<td>0.44</td>
</tr>
<tr>
<td>Aspirin 325 mg</td>
<td>16 (100%)</td>
<td>16 (100%)</td>
<td>1</td>
</tr>
<tr>
<td>CYP2C19*2 heterozygous</td>
<td>8 (50.0%)</td>
<td>4 (25.0%)</td>
<td>0.14</td>
</tr>
<tr>
<td>CYP2C19*2 homozygous</td>
<td>0</td>
<td>2 (12.5%)</td>
<td></td>
</tr>
<tr>
<td>Chronic clopidogrel use, &gt;7 days</td>
<td>8 (50.0%)</td>
<td>6 (37.5%)</td>
<td>0.47</td>
</tr>
<tr>
<td>Platelet reactivity on day 0 (area under the curve/min)</td>
<td>576.0±97.2</td>
<td>573.3±87.1</td>
<td>0.93</td>
</tr>
</tbody>
</table>

### Table 2. Pharmacodynamic Results

<table>
<thead>
<tr>
<th></th>
<th>Patients Randomized to Prasugrel Followed by High-Dose Clopidogrel (n=16)</th>
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<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline platelet reactivity (AUC/min)</td>
<td>576.0±97.2</td>
<td>573.3±87.1</td>
<td>0.957</td>
</tr>
<tr>
<td>Platelet reactivity after 15 days of therapy (AUC/min)</td>
<td>325.8±104.7</td>
<td>478.5±208.5</td>
<td>0.028</td>
</tr>
<tr>
<td>Inhibition of platelet aggregation</td>
<td>49.7±42.9%</td>
<td>9.3±5.2%</td>
<td>0.036</td>
</tr>
<tr>
<td>Difference in platelet reactivity from baseline to day 15 (AUC/min)</td>
<td>251.2±102.1</td>
<td>94.5±150.6</td>
<td>0.002</td>
</tr>
</tbody>
</table>

AUC indicates area under the curve.
high-dose clopidogrel in patients with HTPR 12 to 24 hours after PCI was studied in the GRAVITAS (Gauging Responsiveness With a VerifyNow Assay–Impact on Thrombosis and Safety) trial. The primary outcome of cardiovascular death, myocardial infarction, or stent thrombosis occurred in 2.3% of those treated with high-dose clopidogrel versus 2.3% of the standard-dose group. A significantly higher antiplatelet effect with prasugrel compared with high-dose clopidogrel has been reported in aspirin-treated patients with coronary artery disease, in stable patients undergoing planned PCI, and in acute coronary syndrome patients. Although there are no clinical data recommending a specific goal of therapy to convert nonresponders into responders, the use of prasugrel seems to be an attractive choice to overcome HTPR. Switching from clopidogrel to prasugrel maintenance therapy results in further reductions in maximal ADP-induced platelet aggregation.

Our results are indeed supportive of these prior findings, and confirm that more than one third of such stable PCI patients may exhibit HTPR with standard-dose clopidogrel. The most likely explanation of this phenomenon is genetic variation of CYP2C19, which is implicated in the metabolism of clopidogrel. It has been largely demonstrated that the loss of function of CYP2C19*2 allele is involved in HTPR. In our experience, up to half the patients with HTPR showed a genotype variation in terms of the presence of 1 or 2 copies of the CYP2C19*2 allelic variant. In fact, noncarriers, after testing with either drug, showed a similar response before and after crossover. Finally, we formally appraised the relationship between AUC after treatment with the study drugs and the genotype assessment for the CYP2C19*2 variation. The ensuing receiver-operating characteristic curve identified 600 as the AUC cutoff value, with a 75% sensibility and a 72% specificity.

Despite the abovementioned findings, it should be borne in mind that the correlation between genotype assessment, HTPR, and clinic events remains uncertain, and that 3 pertinent meta-analyses has provided disparate conclusions. In 2010, Mega et al showed that carriage of even 1 reduced-function CYP2C19 allele seemed to be associated with a significantly increased risk of major adverse cerebrovascular events, particularly stent thrombosis, whereas in 2011, Holmes et al concluded that overall there was no significant association between genotype with cardiovascular events. Zabalza et al found that the different results of the previous meta-analysis mainly derived from high heterogeneity between studies analyzing the relationship between CYP2C19 loss-of-function alleles and major cardiovascular outcomes, likely resulting from publication bias. Finally in the Testing Platelet Reactivity In Patients Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy With Prasugrel (TRIGGER-PCI) trial switching from clopidogrel to prasugrel in patients with HTPR afforded effective platelet inhibition. However, given the low rate of adverse ischemic events after PCI with contemporary drug-eluting stents in stable coronary artery disease, the clinical utility of this strategy could not be demonstrated. In fact, the study was stopped prematurely because of a lower-than-expected incidence of the primary end point, thus the clinical utility of this strategy could not be demonstrated.

**Limitations**

This work has several limitations, including the small sample, open-label design, and lack of washout periods. Notably, even a 150-mg clopidogrel dose might not be enough to overcome resistance in some patients. In addition, as the assessment of platelet function inhibition by clopidogrel is highly test specific, our results apply mainly to the method we used. Moreover, the gain-of-function CYP2C19*17 allele was not tested. Finally, the present study was not powered to detect
differences in clinical efficacy or safety between prasugrel or high-dose clopidogrel and was not designed to be powered for an additive genetic model that would be expected to be the pharmacokinetic-appropriate model.

Conclusions
The RESET GENE trial demonstrated that, in HTPR patients, double dose (150 mg) of clopidogrel, although reducing the platelet function, is less effective than prasugrel standard dose. Moreover, despite fact that the study was not powered for an additive genetic model, it showed that prasugrel is more effective in CYP2C19*2 carriers. Finally, receiver-operating characteristic curve analysis could identify the CYP2C19*2 carriers.

Our findings need to be confirmed by further clinical studies.

Disclosures
None.

References


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