Dosing Clopidogrel Based on CYP2C19 Genotype and the Effect on Platelet Reactivity in Patients With Stable Cardiovascular Disease

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Context  Variants in the CYP2C19 gene influence the pharmacologic and clinical response to the standard 75-mg daily maintenance dose of the antiplatelet drug clopidogrel.

Objective  To test whether higher doses (up to 300 mg daily) improve the response to clopidogrel in the setting of loss-of-function CYP2C19 genotypes.

Design, Setting, and Patients  ELEVATE-TIMI 56 was a multicenter, randomized, double-blind trial that enrolled and genotyped 333 patients with cardiovascular disease across 32 sites from October 2010 until September 2011.

Interventions  Maintenance doses of clopidogrel for 4 treatment periods, each lasting approximately 14 days, based on genotype. In total, 247 noncarriers of a CYP2C19*2 loss-of-function allele were to receive 75 and 150 mg daily of clopidogrel (2 periods each), whereas 86 carriers (80 heterozygotes, 6 homozygotes) were to receive 75, 150, 225, and 300 mg daily.

Main Outcome Measures  Platelet function test results (vasodilator-stimulated phosphoprotein [VASP] phosphorylation and VerifyNow P2Y12 assays) and adverse events.

Results  With 75 mg daily, CYP2C19*2 heterozygotes had significantly higher on-treatment platelet reactivity than did noncarriers (VASP platelet reactivity index [PRI]: mean, 70.0%; 95% CI, 66.0%-74.0%; vs 57.5%; 95% CI, 55.1%-59.9%; and VerifyNow P2Y12 reaction units [PRU]: mean, 225.6; 95% CI, 207.7-243.4, vs 163.6; 95% CI, 154.4-173.9; \( P < .001 \) for both comparisons). Among CYP2C19*2 heterozygotes, doses up to 300 mg daily significantly reduced platelet reactivity, with VASP PRI decreasing to 48.9% (95% CI, 44.6%-53.2%) and PRU to 127.5 (95% CI, 109.9-145.2) (\( P < .001 \) for trend across doses for both). Whereas 52% of CYP2C19*2 heterozygotes were nonresponders (\( \geq 230 \) PRU) with 75 mg of clopidogrel, only 10% were nonresponders with 225 or 300 mg (\( P < .001 \) for both).

Clopidogrel, 225 mg daily, reduced platelet reactivity in CYP2C19*2 heterozygotes to levels achieved with standard clopidogrel, 75 mg, in noncarriers (mean ratios of platelet reactivity, VASP PRI, 0.92; 90% CI, 0.85-0.99; and PRU, 0.94; 90% CI, 0.84-1.04). In CYP2C19*2 homozygotes, even with 300 mg daily of clopidogrel, mean VASP PRI was 68.3% (95% CI, 44.9%-91.6%) and mean PRU, 287.0 (95% CI, 170.2-403.8).

Conclusion  Among patients with stable cardiovascular disease, tripling the maintenance dose of clopidogrel to 225 mg daily in CYP2C19*2 heterozygotes achieved levels of platelet reactivity similar to that seen with the standard 75-mg dose in noncarriers; in contrast, for CYP2C19*2 homozygotes, doses as high as 300 mg daily did not result in comparable degrees of platelet inhibition.

Trial Registration  clinicaltrials.gov Identifier: NCT01235351

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CLOPIDOGREL DOSES AND CYP2C19 GENOTYPE

Figure 1. Patient Flow Diagram

The number of individuals screened for eligibility is not known.

source of the variable response to the antiplatelet medication.

Cytochrome P450 (CYP) enzymes play a critical role in the metabolism of clopidogrel. Multiple studies have demonstrated that both heterozygotes and homozygotes for loss-of-function CYP2C19 alleles have lower levels of the active clopidogrel metabolite, \(^{6,7}\) diminished platelet inhibition, \(^{8,9}\) and higher rates of adverse cardiovascular events as compared with noncarriers in the setting of treatment with standard 75-mg maintenance doses of clopidogrel. \(^{10}\) Regulatory agencies, including the US Food and Drug Administration (FDA) and the European Medicines Agency, have responded to these data. In the case of the FDA, a boxed warning was added to the clopidogrel prescribing information that recommends considering alternative treatment strategies in patients with particular CYP2C19 genotypes.

As such, data are needed to offer guidance as to what might constitute optimal treatment strategies in patients with loss-of-function CYP2C19 alleles. Although newer P2Y\(_{12}\) inhibitors are available that are unaffected by CYP2C19 genotype, these medications can be expensive and are not accessible in all countries. In contrast, clopidogrel is widely available, already generic in some countries, and anticipated to be generic in the United States in 2012, which may influence the cost.

Therefore, we conducted ELEVATE-TIMI 56, a multicenter, randomized, double-blind trial, to test whether maintenance doses of up to 300 mg daily of clopidogrel can improve platelet reactivity in the setting of the major loss-of-function CYP2C19 genotype (CYP2C19*2), particularly among heterozygotes, who constitute approximately 25% to 45% of the population depending on racial background. We hypothesized that: (1) increasing the maintenance doses of clopidogrel in patients who carry a CYP2C19*2 allele will correspondingly reduce platelet reactivity, and (2) among carriers of CYP2C19*2, a higher maintenance dose of clopidogrel can be found that reduces platelet reactivity to the levels achieved in noncarriers treated with the established, standard 75-mg daily maintenance dose of clopidogrel.

METHODS

In total, 335 patients from 32 sites were enrolled from October 2010 until September 2011 in the trial, which was conducted in patients with known cardiovascular disease taking 75 mg of clopidogrel daily (FIGURE 1). To be eligible, patients needed to have an indication for the use of clopidogrel (either a myocardial infarction [MI] and/or PCI ≥ 4 weeks and ≤ 6 months prior to enrollment) and be clinically stable. All patients took aspirin, 81 to 325 mg, daily, and they were requested to keep taking a stable dose during the study if medically indicated. Key exclusion criteria were use of anticoagulants or proton pump inhibitors (PPIs), current smoking, prior stent thrombosis,
heightened risk of bleeding, end-stage renal or hepatic disease, or a procedure or hospitalization planned in the next 12 weeks.

**Study Protocol**

At the time of enrollment, a baseline clinical evaluation was performed, and each patient underwent blood sampling for blinded genotyping and platelet function testing. A pyrosequencing assay was performed at the Center for Advanced Molecular Diagnostics at Brigham and Women’s Hospital to assess for CYP2C19*2 status (National Center for Biotechnology Information [NCBI] genome build 37.1, NG_008384). Genotyping was repeated using a Nanosphere Verigene 2C19/CBS nucleic acid research-use-only assay. A total of 333 individuals were successfully genotyped and confirmed for CYP2C19*2 status.

Patients were allocated, using a central interactive voice response system (Worldwide Clinical Trials), to a blinded sequence of maintenance doses of clopidogrel for 4 treatment periods, each approximately 14 (±3) days. Patients who were carriers of CYP2C19*2 received 75, 150, 225, and 300 mg daily of clopidogrel (in various sequences). Noncarriers received 75 and 150 mg daily (2 periods of each dose) of clopidogrel (in various sequences). At the end of each treatment period, platelet function testing was performed and ischemic, bleeding, and other adverse events were ascertained. After the last study drug treatment period, patients resumed their prestudy antiplatelet medication if indicated and were contacted approximately 30 days later to assess for any adverse events.

The trial was approved by the institutional review board of each participating site, and participants provided written informed consent. An independent data and safety monitor reviewed unblinded data.

**End Points**

The outcome measurement was on-treatment platelet reactivity index (PRI) determined through flow cytometric assessment of phosphorylation status of vasodilator-stimulated phosphoprotein (VASP). The VASP PRI was determined from ambient blood samples sent to the Center for Platelet Research Studies (Children’s Hospital Boston), which was blinded to patient treatment group. Platelet function testing was also conducted at each site with an encrypted point-of-care device (VerifyNow P2Y12 test; Accutronics) and reported as P2Y12 reaction units (PRU), indicating the amount of ADP-mediated platelet aggregation specific to the platelet P2Y12 receptor. Nonresponder status was pre-specified at 230 PRU or greater. Based on data that emerged after initiation of ELEVATE-TIMI 56, an additional cut point of 208 PRU or greater was also explored. Fatal, cardiovascular, cerebrovascular, and bleeding events were also assessed at each visit. These events were adjudicated by a blinded clinical event adjudicator. Adverse events and serious adverse events were collected.

**Statistical Analysis**

Continuous data are presented as means and 95% confidence intervals, and categorical data are presented as counts and percentages unless otherwise specified. The analytic data set consisted of all patients who were successfully genotyped, with the exception of 1 patient who was ultimately found to have a non-*2 loss-of-function CYP2C19 allele (and therefore never received the 75 mg of clopidogrel). No imputation was applied for missing data, and platelet function testing was analyzed per-protocol.

The association of different doses of clopidogrel (test of trend) with platelet reactivity among carriers (with heterozygotes and homozygotes tested separately and combined) and noncarriers of the CYP2C19*2 loss-of-function allele was evaluated by fitting a repeated mixed model with patient as a random effect and doses as fixed effects, and, in the case of VASP PRI, adjusting for the time from sample collection to analysis. No significant carryover effects were detected for the dose sequences among CYP2C19*2 noncarriers (P=.82) or carriers (P=.46). Least square means and the differences among the means were calculated. Pairwise differences among means (ie, between individual clopidogrel maintenance doses) were assessed. Tukey-Kramer correction for multiple testing was used. Proportions of non-responders were compared within each genotype group using a generalized linear model to account for correlated observations. A 2-sided P value of less than .05 was set as the level of significance.

The least square means from the mixed models were used to test whether higher maintenance doses of clopidogrel in carriers of a loss-of-function CYP2C19 allele could result in similar platelet inhibition as compared with a standard maintenance dose of clopidogrel in noncarriers. Differences among the least square means were calculated and reported with the 95% confidence intervals. Ratios of the means were calculated with 90% confidence intervals, and equivalence boundaries of 0.8 to 1.25 were used, extrapolating from prior FDA guidance.

To obtain at least 80% power to detect a 7.5% difference in the paired VASP PRI means of the tested doses (which corresponds to 50% of the observed difference seen with increasing the maintenance dose of clopidogrel from 75 to 150 mg in patients of unknown genotype), and maintaining an overall α of .05 for the study, at least 76 CYP2C19 loss-of-function allele carriers were required. In this calculation, the standard deviation of the difference was assumed to be 20%. Based on an estimate of the proportion of patients carrying a CYP2C19 loss-of-function allele, the total sample size needed to be at least 254.

The following clinical outcomes were assessed by dose for CYP2C19*2 carriers and noncarriers using an intention-to-treat approach: death (cardiovascular and noncardiovascular), cardiac ischemic events (MI, unstable angina, urgent coronary revascularization), cerebrovascular events (stroke and transient ischemic attack), and bleeding (Thrombolysis in Myocardial Infarction [TIMI] scales). Analyses were conducted using SAS version 9.2.
**RESULTS**

In total, 333 patients were enrolled and underwent genotyping. Their mean (SD) age was 60.2 (9.9) years, 74.8% were male, 57.1% had a history of MI, and 97.3% had a history of PCI. A total of 247 patients were noncarriers of a CYP2C19*2 allele whereas 86 were carriers (80 heterozygotes and 6 homozygotes). Clinical characteristics did not differ between the carrier and noncarrier groups (Table 1). The adherence rates for the 75-, 150-, 225-, and 300-mg doses of clopidogrel among CYP2C19*2 carriers on study drug were 97.3%, 98.1%, 98.6%, and 98.3%, respectively.

**Table 1. Patient Baseline Characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall (N = 333)</th>
<th>CYP2C19*2 Noncarriers (n = 247)</th>
<th>CYP2C19*2 Carriers (n = 86)</th>
<th>P Value (Carriers vs Noncarriers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>60.2 (9.9)</td>
<td>60.8 (9.9)</td>
<td>58.6 (9.7)</td>
<td>.07</td>
</tr>
<tr>
<td>Male sex</td>
<td>249 (74.8)</td>
<td>186 (73.3)</td>
<td>63 (73.3)</td>
<td>.71</td>
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<tr>
<td>Race/ethnicityb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>293 (88.0)</td>
<td>224 (90.7)</td>
<td>69 (80.2)</td>
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<tr>
<td>Black</td>
<td>30 (9.0)</td>
<td>18 (7.3)</td>
<td>12 (14.0)</td>
<td>.02</td>
</tr>
<tr>
<td>Asian</td>
<td>8 (2.4)</td>
<td>3 (1.2)</td>
<td>5 (5.8)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>2 (0.6)</td>
<td>2 (0.8)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure, mean (SD), mm Hg</td>
<td>126.8 (14.8)</td>
<td>126.6 (13.8)</td>
<td>127.3 (18.3)</td>
<td>.67</td>
</tr>
<tr>
<td>Diastolic blood pressure, mean (SD), mm Hg</td>
<td>76.0 (9.4)</td>
<td>75.4 (9.3)</td>
<td>77.5 (9.7)</td>
<td>.12</td>
</tr>
<tr>
<td>Heart rate, mean (SD), /min</td>
<td>68.6 (10.6)</td>
<td>68.4 (10.4)</td>
<td>69.1 (11.2)</td>
<td>.38</td>
</tr>
<tr>
<td>BMI, mean (SD)c</td>
<td>30.7 (6.3)</td>
<td>30.4 (5.5)</td>
<td>31.4 (8.1)</td>
<td>.93</td>
</tr>
<tr>
<td>Hypertension</td>
<td>287 (86.2)</td>
<td>214 (86.6)</td>
<td>73 (84.9)</td>
<td>.68</td>
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<tr>
<td>Hypercholesterolemia</td>
<td>315 (94.6)</td>
<td>232 (93.9)</td>
<td>83 (96.5)</td>
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<td>Diabetes mellitus</td>
<td>118 (35.4)</td>
<td>90 (36.4)</td>
<td>28 (32.6)</td>
<td>.52</td>
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<tr>
<td>Family history of CAD</td>
<td>137 (41.1)</td>
<td>99 (40.1)</td>
<td>38 (44.2)</td>
<td>.51</td>
</tr>
<tr>
<td>History of MI</td>
<td>190 (57.1)</td>
<td>134 (54.3)</td>
<td>56 (65.1)</td>
<td>.08</td>
</tr>
<tr>
<td>History of PCI</td>
<td>324 (97.3)</td>
<td>240 (97.2)</td>
<td>84 (97.7)</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>CAGB procedure</td>
<td>59 (17.7)</td>
<td>43 (17.4)</td>
<td>16 (18.6)</td>
<td>.80</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; CAGB, coronary artery bypass graft; CAD, coronary artery disease; MI, myocardial infarction; PCI, percutaneous coronary intervention.

Response to Higher Doses of Clopidogrel

When treated with a standard clopidogrel maintenance dose of 75 mg daily, both CYP2C19*2 heterozygotes and homozygotes had significantly higher on-treatment platelet reactivity than did noncarrier patients (mean VASP PRI, 70.0%; 95% CI, 66.0%-74.0%, and 86.6%; 93% CI, 80.7%-92.3%, vs 57.5%; 95% CI, 55.1%-59.9%; P < .001 for both comparisons). Among CYP2C19*2 heterozygotes, higher clopidogrel maintenance doses (up to 300 mg) produced significant reductions in platelet reactivity (P < .001 for trend) (Figure 2 and Table 2). Each 75-mg more of clopidogrel daily resulted in an approximate 8% to 9% absolute reduction in VASP PRI, with some attenuation between the 2 highest doses. Moreover, individual pairwise comparisons between the higher daily doses of clopidogrel (150, 225, and 300 mg) and the 75-mg daily dose of clopidogrel were all statistically significant (P < .001) as were comparisons between each of the higher doses (P < .001) except for the comparison between 225 and 300 mg daily.

The VerifyNow results as assessed by PRU were similar to the VASP data across dose and genotype (Table 2). When treated with the standard maintenance dose of clopidogrel, 75-mg daily, both CYP2C19*2 heterozygotes and homozygotes had significantly higher platelet reactivity than did noncarrier patients (mean PRU, 225.6; 95% CI, 207.7-243.4, and 328.8; 95% CI, 247.9-409.7, vs 163.6; 95% CI, 154.4-173.9; P < .001 for both comparisons). Analogous to the VASP findings, higher doses of clopidogrel resulted in a significant reduction in platelet reactivity among CYP2C19*2 heterozygotes (P < .001 for trend).

Using a prespecified cut point for non-responder status (defined as on-treatment platelet reactivity ≥230 PRU), 33 of 234 noncarriers (23%) were non-responders with the 75-mg dose and 28 of 227 (12%) with the 150-mg dose. Among CYP2C19*2 heterozygotes, 40 of 76 (52%) were nonresponders with the
75-mg dose of clopidogrel. Higher maintenance doses of clopidogrel in CYP2C19*2 heterozygotes significantly reduced the proportion of nonresponders to 10% with 225 mg daily (8/75, P < .001) and 300 mg daily (7/73, P < .001) (Figure 3). The risk ratios for nonresponder status were 0.51 (95% CI, 0.37-0.69), 0.21 (95% CI, 0.11-0.39), and 0.20 (95% CI, 0.11-0.38) with 150, 225, and 300 mg daily of clopidogrel, respectively. Among the CYP2C19*2-heterozygote nonresponders to clopidogrel, 75 mg daily, the proportions who became responders were 49% (18/37), 79% (31/39), and 81% (30/37) when treated, respectively, with 150, 225, and 300 mg daily of clopidogrel. Similar results were obtained when a cut point for nonresponder status of 208 PRU or greater was used (eFigure 1, available at http://www.jama.com).

When evaluating the CYP2C19*2 homozygotes, we saw a trend toward less platelet reactivity with higher maintenance doses of clopidogrel; however, even with 300 mg daily of clopidogrel, these individuals had a mean VASP PRI of 68.3% (95% CI, 44.9%-91.6%) and VerifyNow PRU of 287.0 (95% CI, 170.2-403.8). Similarly, the nonresponder rate (≥230 PRU) was 80% (4/5) with clopidogrel, 75 mg, daily and remained high at 60% (3/5) despite clopidogrel, 300 mg, daily.

**High-Dose Clopidogrel in CYP2C19*2 Carriers vs Standard-Dose Clopidogrel in Noncarriers**

In CYP2C19*2 heterozygotes, a 150-mg daily maintenance dose of clopidogrel still resulted in platelet reactivity that tended to be higher than that seen in noncarrier patients treated with 75 mg daily (Figure 4). In contrast, the 225-mg daily dose resulted in platelet reactivity at least as good as what was observed in response to standard clopidogrel dosing in noncarrier patients (mean ratios of platelet reactivity, VASP PRI, 0.92; 90% CI, 0.85-0.99, and PRU, 0.94; 90% CI, 0.84-1.04) (eFigure 2). A 300-mg daily dose of clopidogrel resulted in superior reductions in platelet reactivity as compared with standard 75-mg clopidogrel dosing in noncarrier patients as measured both by VASP PRI and PRU (P < .001 for both). For CYP2C19*2 homozygotes, even 300 mg daily of clopidogrel did not result in platelet reactivity levels similar to standard clopidogrel dosing in noncarriers (Table 2).

**Clinical Events**

There were no deaths, cerebrovascular events, or TIMI major or minor bleeding events. Among CYP2C19*2 carriers, 55.5% had a lower VerifyNow PRU of 287.0 (95% CI, 44.9%-91.6%) and 50.1 (45.9%-54.3) with 150, 225, and 300 mg daily of clopidogrel, 75 mg, daily, the proportions who became responders were 49% (18/37), 79% (31/39), and 81% (30/37) when treated, respectively, with 150, 225, and 300 mg daily of clopidogrel. Similar results were obtained when a cut point for nonresponder status of 208 PRU or greater was used (eFigure 1, available at http://www.jama.com).

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noncarriers taking the 75 mg and 150 mg doses, 2 patients had cardiac ischemic events while taking the 75-mg dose and 3 with the 150-mg dose. There were 0 and 5 patients with bleeding events, respectively, that met criteria for bleeding requiring medical attention. Among carriers of a CYP2C19*2 allele, there was 1 patient who experienced a cardiac ischemic event at the 75-mg dose of clopidogrel. A bleeding event requiring medical attention occurred in 1 of the patients in each of the following dosing regimens: 75, 225, and 300 mg. No adverse events or serious adverse events that were not end points occurred at a frequency greater than 3% per treatment group, and there were no significant imbalances specifically for hematologic, gastrointestinal, or musculoskeletal disorders for CYP2C19*2 carriers at the different doses (eTable).

**COMMENT**

Clopidogrel, one of the most commonly prescribed cardiovascular medications in the world, is approved for a single maintenance dose of 75 mg daily. However, the response to clopidogrel is variable, and as a group, patients with either 1 or 2 loss-of-function CYP2C19 alleles have an attenuated pharmacologic response and worse clinical outcomes in the setting of treatment with the standard dose of clopidogrel. We now show that (1) higher maintenance doses of clopidogrel in patients carrying a CYP2C19*2 allele significantly reduce platelet reactivity, and (2) daily maintenance doses of 225 mg of clopidogrel or greater in CYP2C19*2 heterozygotes can achieve on-treatment platelet reactivity at least comparable with what is achieved with 75 mg daily of clopidogrel in noncarrier patients with cardiovascular disease.

Prior systematic pharmacogenetic studies have tested clopidogrel maintenance doses of 150 mg daily in carriers of a loss-of-function CYP2C19 allele and have reported mixed results. In one study of healthy individuals, a 150-mg maintenance dose of clopidogrel adequately reduced platelet reactivity in carriers of 1 or 2 loss-of-function CYP2C19 alleles, other studies conducted in patients with cardiovascular disease with CYP2C19 loss-of-function alleles have not demonstrated as significant reductions in platelet reactivity with the 150-mg dose of clopidogrel. Concordant with the latter observation, we found that increasing the maintenance dose from 75 to 150 mg did not on average inhibit platelet reactivity to the levels seen with 75 mg of clopidogrel in noncarrier patients. Rather, we found that on average, it required a tripling of the clopidogrel maintenance dose to achieve platelet reactivity that is at least as good as that observed with 75 mg of clopidogrel in CYP2C19*2 noncarriers. Interestingly, CLOVIS-2 studied the pharmacologic efficacy of different loading doses of clopidogrel based on CYP2C19 genotype and also found that a tripling of the dose (ie, a 900-mg load) achieved a degree of platelet inhibition comparable with what was observed in response to the standard 300-mg loading dose in noncarrier patients. Patients in our trial tolerated these higher maintenance doses of clopidogrel during the approximately 2-week duration of each therapy, with low rates of discontinuation. However, further long-term intervention trials are warranted.

Prasugrel and ticagrelor are newer P2Y12 ADP receptor blockers that achieve significantly higher levels of platelet inhibition than does clopidogrel. These agents have been shown to reduce ischemic events as compared with clopidogrel in patients with ACS, although with increased rates of nonsurgical bleeding. In contrast to clopidogrel, the effects of prasugrel and ticagrelor are unaffected by variants in the CYP2C19 gene (and correspondingly, no association between CYP2C19 genotype and clinical events has been observed in patients treated with these agents) and thus these medications represent other alternative treatment strategies in such patients. However, clopidogrel will likely continue to be used throughout the world, and it is therefore important to understand clopidogrel’s effects across CYP2C19 genotypes and with higher maintenance doses.

Studies have evaluated whether tailoring clopidogrel therapy using platelet function testing improves clinical outcomes. Bonello and colleagues gave patients repeated loading doses of clopidogrel (up to 2400 mg total) to achieve a target level of suppression of platelet

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**Figure 4. Difference in Platelet Reactivity Between CYP2C19*2 Heterozygotes Treated With Increasing Doses of Clopidogrel With Noncarriers Treated With 75 mg of Clopidogrel Daily**

Data are reported as least squares differences and 95% confidence intervals for platelet reactivity between CYP2C19*2 heterozygotes at clopidogrel doses of 75 mg (n=76), 150 mg (n=73), 225 mg (n=75), and 300 mg (n=73) and noncarriers at 75 mg of clopidogrel (n=237). Differences in least squares means were tested using asymptotic methods (normal z test). VASP indicates vasodilator-stimulated phosphoprotein phosphorylation assay.
reactivity in each patient before PCI, and this strategy resulted in a significant decrease in adverse outcomes. In contrast, in GRAVITAS and RECLOSE 2-ACS, higher maintenance doses of clopidogrel after PCI in patients with high on-treatment platelet reactivity at baseline did not improve outcomes. The higher maintenance dose in GRAVITAS was clopidogrel, 150 mg, daily, which resulted in a median PRU of 211 (interquartile range, 155-262), and therefore somewhere between 25% and 50% of the population remained nonresponders with this dose. In RECLOSE 2-ACS, despite treating with higher maintenance doses of clopidogrel or switching to ticlopidine, 38% of patients continued to be nonresponders. ELEVATE-TIMI 56, in conjunction with the important observations from GRAVITAS and RECLOSE 2-ACS, highlights that doses even higher than 150 mg of clopidogrel or other agents may be necessary when tailoring antiplatelet therapy to overcome resistance in some patients, particularly those who carry a loss-of-function CYP2C19 allele.

Patients who carry 2 CYP2C19 loss-of-function alleles, also known as poor metabolizers, face particular hurdles when it comes to generating enough active clopidogrel metabolite and platelet inhibition with standard doses of clopidogrel, as noted by the FDA. ELEVATE-TIMI 56 was not specifically powered to evaluate these individuals, as they constitute only 2% of the tested population. Nonetheless, in ELEVATE-TIMI 56, among the subset of CYP2C19*2 homozygous patients, even the 300-mg dose of clopidogrel was not able to achieve on-treatment platelet reactivity comparable with clopidogrel, 75 mg, daily in noncarriers. Similarly, in CLOVIS-2, the high clopidogrel loading dose of 900 mg was able to adequately lower on-treatment platelet reactivity among carriers of 1 loss-of-function CYP2C19 allele but not 2. Such patients may be better served by treatment with prasugrel or ticagrelor, or perhaps the addition of clostazol, all of which do not appear to be affected significantly by the CYP2C19 variants.

There are some limitations to these analyses. First, treatment allocation was based on carriage of only the *2 CYP2C19 loss-of-function allele, for which we could engage in timely, validated genotyping. More comprehensive genotyping panels are available; of note though, CYP2C19*2 is the most frequent variant, accounting for greater than 95% of the loss-of-function allele carrier status. Second, each treatment period consisted of approximately 14 days. This time frame allowed for a reasonable window to achieve steady state antiplatelet effect with each regimen with no significant carryover effect from the prior treatment. However, defining the long-term tolerability of the higher-dose regimens will require gathering additional experience. Third, PPI use at baseline was an exclusion criterion. Whereas this approach allowed for a focused evaluation of higher doses of clopidogrel in patients with CYP2C19 genetic variants, further studies will be needed to address questions pertaining to possible interactions between clopidogrel dosing, PPI use, and genotype. Fourth, this study focused on achieved platelet reactivity as the primary outcome of interest. While platelet reactivity is a well-validated predictor of poor clinical outcomes, large-scale and long-term trials powered for clinical outcomes will be necessary to assess for adverse events and establish preferred treatment regimens. Until then, ELEVATE-TIMI 56 offers a framework to rationally select alternative doses and lays the foundation for future studies.

In conclusion, ELEVATE-TIMI 56 demonstrates that among patients with stable cardiovascular disease, tripling the maintenance dose of clopidogrel to 225 mg daily in CYP2C19*2 heterozygotes achieved levels of platelet reactivity similar to that seen with the standard 75-mg dose in noncarriers. Even 300 mg of clopidogrel daily, however, is unlikely to result in optimal degrees of platelet inhibition in CYP2C19*2 homozygotes. These data help define how patients with different CYP2C19 genotypes respond to clopidogrel maintenance dosing strategies and provides useful information to guide further clinical studies.

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REFERENCES


