Translational platelet research in patients with coronary artery disease: What are the major knowledge gaps?

Paul A. Gurbel1; Matthew T. Roe2; Joseph A. Jakubowski3; Svatli Shah2; David Erlinge4; Shaun G. Goodman5; Kurt Huber6; Mark Y. Chan7; Jan H. Cornel8; Udaya S. Tantry1; E. Magnus Ohman1

1Sinai Center for Thrombosis Research, Baltimore, Maryland, USA; 2Duke University Medical Center, Durham, North Carolina, USA; 3Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, USA; 4Department of Cardiology, Lund University, Lund, Sweden; 5Canadian Heart Research Centre and St. Michael’s Hospital, University of Toronto, Toronto, Ontario, Canada; 6Medizinische Abteilung (Kardiologie), Wilhelminenspital, Vienna, Austria; 7National University Heart Centre, Singapore, Singapore; 8Medisch Centrum Alkmaar, Alkmaar, Netherlands

Summary
Translational platelet function investigations performed in the percutaneous coronary intervention (PCI)-treated population receiving clopidogrel have identified high platelet reactivity to ADP (HPR) as a major risk factor for both acute as well as long-term ischaemic event occurrence, including stent thrombosis. Recent studies have highlighted the relation of single nucleotide polymorphisms of genes involved in clopidogrel absorption and metabolism to reduced pharmacokinetic and pharmacodynamic responses to clopidogrel. CYP2C19 loss-of-function (LoF) allele carriage has been associated with increased thrombotic risk in the PCI population. However, there is no information regarding the utility of platelet function testing to predict outcomes in patients with stable coronary artery disease and in medically managed patients with acute coronary syndromes. Additionally, few studies have included longitudinal assessment of platelet function to assess a potential time-dependent relation to ischaemic event occurrence and no phase-III antiplatelet-therapy trial has included a large enough platelet function sub-study to examine the relation between on-treatment platelet reactivity, bleeding, and ischaemic event occurrence. Therefore, further studies are needed to delineate the role of platelet function testing across the spectrum of symptomatic coronary artery disease.

Keywords
ADP receptors, clinical trials, antiplatelet drugs, platelet pharmacology, coronary syndrome

Introduction
Evidence exists that platelet-rich thrombus formation induced by plaque rupture and endothelial cell erosion is the primary cause of ischaemic event occurrence in patients with acute coronary syndromes (ACS) (1). Thromboxane A2 (TXA2) and adenosine diphosphate (ADP) are major agonists released from activated platelets. The P2Y12 receptor plays a critical role in amplifying platelet activation in response to numerous agonists and the ADP-P2Y12 interaction is central to the genesis of thrombosis (2). Large-scale trials have demonstrated that the combination of clopidogrel and aspirin therapy, commonly known as dual antiplatelet therapy (DAPT), significantly reduced the incidence of the composite end-point of death, myocardial infarction (MI), and stroke in a wide range of patients with ACS compared to aspirin monotherapy (3). Based on these data, patients with ACS are largely treated with a non-selective or “one-size-fits-all” approach without any assessment of antiplatelet response, despite the fact that clopidogrel therapy is associated with response variability and resistance (4).

Furthermore, although the mechanism(s) of recurrent ischaemic event occurrence (~10% prevalence) and major bleeding (~2% prevalence) during DAPT are of tremendous clinical importance, large scale clinical trials have not included adequately sized sub-studies to determine the relation of treatment failure (ischaemia and bleeding) to on-treatment platelet reactivity (4).

Rationale for genetic testing
Multiple lines of evidence suggested that variable and insufficient active metabolite generation were the primary explanations for clopidogrel response variability and non-responsiveness, respectively (5). Clopidogrel, a prodrug, is converted to an active metabolite by hepatic cytochrome (CYP) P450 isoenzymes. CYP isoenzyme activity is influenced by single nucleotide polymorphisms (SNPs) and other drugs. These drugs compete with clopidogrel for CYP-mediated metabolism or inhibit the CYP
isoenzymes involved in clopidogrel metabolism. Candidate gene studies conducted in healthy volunteers demonstrated that loss-of-function (LoF) polymorphisms of CYP2C19 were associated with decreased clopidogrel active metabolite exposure and less platelet inhibition (6, 7). In the first genome-wide association study, conducted in healthy subjects, CYP2C19*2 was the only SNP significantly associated with clopidogrel response variability. In a replication study of percutaneous coronary intervention (PCI)-treated patients, carriers of the LoF CYP2C19*2 allele had ~2.4x higher cardiovascular event occurrence compared with non-carriers (8). In a collaborative meta-analysis of trials primarily involving PCI-treated patients, carriers of the CYP2C19*2 allele had 2.4x higher cardiovascular event occurrence compared with non-carriers (9).

Subsequent retrospective analyses of ACS trials involving mainly a mix of PCI and medically treated patients failed to demonstrate a significant association between CYP2C19 LoF allele carriage and adverse clinical outcomes [Fig. 1, (10, 11)]. The relation of the gain-of-function allele (CYP2C19*17) carrier status and ABCB1 genotype to the antiplatelet response of clopidogrel and clinical outcomes in clopidogrel-treated patients are inconclusive at this time (10–13). The relation of SNPs of the gene encoding paraoxonase-1 (PON-1) to the pharmacokinetic and pharmacodynamic effects of clopidogrel and clinical outcome is controversial (14–16).

In summary, the evidence to this date indicates that LoF allele carrier status is an important independent predictor of the pharmacodynamic response to clopidogrel and appears to influence clinical outcomes in high risk clopidogrel-treated patients who have undergone PCI. However, the relation of LoF allele carrier status to clinical outcomes has never been determined in a trial of a medically managed ACS population.

Use of other antiplatelet agents or alternative dosing strategies of clopidogrel to overcome the influence of the LoF allele has been proposed (17). However, recent evidence indicated that therapy with high maintenance-dose clopidogrel (150 mg daily) was not a highly effective strategy to overcome the influence of the LoF allele and, in poor metabolisers, had no enhanced effect on reducing platelet reactivity as compared to standard dose clopidogrel (75 mg daily) (15, 18).

In a recent prospective multicentre study, among 411 patients with non-ST-segment elevation acute coronary syndrome undergoing PCI, patients carrying CYP2C19*2 and exhibiting high platelet reactivity (HPR) (n=103) after a first 600-mg loading dose of clopidogrel were treated with three repeated 600 mg doses to obtain a vasodilator-stimulated phosphoprotein (VASP) index <50%. With this repeated loading strategy, a <50% VASP phosphorylation level was achieved in 88% of patients (19). In another multicenter, randomised, double-blind trial of 333 patients with cardiovascular disease, tripling the standard maintenance dose of clopidogrel (225 mg daily) in CYP2C 19*2 heterozygotes (n=80) achieved a level of platelet reactivity similar to the level observed in non-carriers treated with the standard dose (75 mg daily). Whereas, in homozygotes (n=6) even a 300 mg daily dose was not sufficient to achieve the same level of platelet reactivity (20). Although the latter studies demonstrated that HPR in CYP2C19*2 carriers can be overcome by repeated clopidogrel loading doses or maintenance doses, both of these approaches are not practical. For example, it is not possible, especially in the United States, to keep patients for a prolonged period of time in the hospital before PCI due to economic reasons, and also this strategy is not practical in high-risk patients. Importantly, the clinical efficacy and safety of prolonged high-dose clopidogrel have never been evaluated. Rather, treating patients with HPR or CYP2C19*2 carriers with prasugrel or ticagrelor is a more practical strategy that has been ex-

![Figure 1: CYP2C19 LOF carrier status has only been linked to risk in the PCI population. *P-value of interaction indicates significance of effects of genotype groups on the results of comparisons between treatment groups.](image-url)
tensively evaluated and is more effective in eliminating HPR (11, 14, 21, 22). In addition, the latter strategy is more economical than performing repeated platelet function tests.

Therapy with the third-generation thienopyridine, prasugrel (60 mg load/10 mg daily maintenance) resulted in a 19% relative reduction in the occurrence of the primary composite endpoint of cardiovascular death, MI and stroke compared to clopidogrel therapy (300 mg load/75 mg daily maintenance) in patients undergoing PCI in the The TRial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet InhibitioN with Prasugrel (TRITON) TIMI-38 trial (23). In a randomised study of patients with stable coronary artery disease (CAD), the CYP2C19 genotype and SNPs of genes encoding other isoenzymes did not affect prasugrel active metabolite formation or the magnitude of platelet inhibition either after loading or during the maintenance phase (24). Furthermore, in a sub-analysis of the TRITON TIMI-38 trial, carriers of the LoF allele treated with clopidogrel had higher rates of the primary outcome and definite/probable stent thrombosis compared to non-carriers whereas among prasugrel-treated patients, LoF carrier status was unrelated to outcomes (Fig. 1) (9, 25).

The CYP2C19 LoF genotype significantly influenced the antiplatelet effect of clopidogrel, but not ticagrelor, a direct-acting P2Y₁₂ receptor blocker, in stable CAD patients (21). In the genetic substudy of the Platelet Inhibition and Patient Outcomes (PLATO) trial, an ACS study where ~64% of patients underwent PCI, ticagrelor was associated with a reduced occurrence of cardiovascular events compared with clopidogrel irrespective of genotype (11). Taken together, these data strongly suggest that prasugrel and ticagrelor are effective alternatives to overcome the influence of the LoF allele carrier state. Currently there are no data from prospective trials specifically designed to assess the clinical efficacy and safety of new P2Y₁₂ receptor blockers in patients identified as LoF allele carriers. However, the ongoing prospective randomised Pharmacogenomics of Anti-platelet Intervention (PAPI)-2 trial will determine the efficacy and safety of clopidogrel vs. prasugrel in *2 carriers undergoing PCI and the Ge- notype Guided Comparison of Clopidogrel and Prasugrel Outcomes Study (GeCCO) study will compare the effectiveness of clopidogrel in CYP2C19 extensive metabolisers (EM) with prasugrel in adults recently hospitalised for ACS with primary, delayed, or planned PCI (NCT#01452152, NCT00998514, respectively).

The fundamental reason for genotyping clopidogrel-treated patients is to identify those at risk of having high risk phenotype, i.e. the patients with HPR. However, as noted above, clopidogrel metabolism is influenced by concomitantly administered drugs and agents that either interact or compete with clopidogrel during hepatic cytochrome P450-mediated metabolism such as proton pump inhibitors, calcium channel blockers, warfarin, old age, and cigarette smoke (Fig. 2) (4, 26–28). Although influence of these drug-drug interactions on clopidogrel pharmacokinetics and pharmacodynamics has been reported based on prospective studies, to date no prospective study has conclusively demonstrated the clinically meaningful influence of these drugs in patients treated with clopidogrel. In addition, it has been recently demonstrated that current cigarette smoking (≥1/2 pack per day) enhances the pharmacodynamic and clinical effects of clopidogrel (28–30). Moreover, it has been reported that on-treatment platelet reactivity to ADP is influenced by the CAD state, age, gender, diabetes and obesity (4). The net effect of all of these influences is reflected in the final platelet reactivity phe-
Rationale for platelet function testing – Determining the therapeutic window

The unpredictable antiplatelet response to clopidogrel in the PCI patient was reported nearly a decade ago; ~30% of PCI patients were non-responders (≤10% decrease in platelet aggregation from baseline) at 24 hours after a 300 mg load and this prevalence of non-responsiveness persisted at five days and fell to ~15% at 30 days post-PCI during 75 mg/day maintenance therapy (33). Similar observations of the relation of clopidogrel response over time were demonstrated in the GRAVITAS (Gauging Responsiveness With A VerifyNow assay-Impact on Thrombosis And Safety) trial (34) and in a recent study by Campo et al. (35).

Following the demonstration of clopidogrel-response variabil-
ity, over 30 translational research studies conducted around the world involving thousands of patients utilising multiple labora-
tory tests have reached the identical conclusion: patients treated with PCI who have HPR are at increased risk for both short-term as well as long-term post-PCI ischaemic event occurrence, including stent thrombosis (4, 36, 37). These studies have primarily used a single measurement of reactivity determined either immediately before PCI or at the time of hospital discharge. A recent consensus statement proposed cut-off values based on receiver-operating characteristic curve (ROC) analysis for different platelet function assays to be used in future studies of personalised antiplatelet therapy (Tables 1 and 2) (4, 35–41, 66). A recent patient-based meta-
analyses of studies employing the VerifyNow point-of-care assay lends further support for the potential role of monitoring of P2Y12

Test | HPR cut-off | PPV | NPV | Reference (year) (ref. no.)
--- | --- | --- | --- | ---
Light Transmittance Aggregometry | | | | |
5 μM ADP-Induced Aggregation | 43% | 12% | 94% | Breet et al. (2010) (66)
20 μM ADP-Induced Aggregation | 65% | 12% | 94% | Breet et al. (2010) (66)
VerifyNow P2Y12 Assay | 236 PRU | 13% | 94% | Breet et al. (2010) (66)
Vasodilator-stimulated Phosphoprotein Phosphorylation Assay | 50% VASP-PRI | 18% | 100% | Bonello et al. (2007) (40)
Multiplate Analyzer | 468 AU x min. | 2.5% | 100% | Sibbing et al. (2009) (41)

ADP = adenosine diphosphate, HPR = high platelet reactivity, NPV = negative predictive value, PPV = positive predictive value, PRU = P2Y12 reaction units, VASP-PRI = Vasodilator Stimulated Phosphoprotein – platelet reactivity index. AU x Min = Arbitrary units x minutes.
alisation of antiplatelet therapy (57). Recent studies have shown evidence to support the concept of a therapeutic window for P2Y_{12} inhibitors (35, 52).

**Tailored anti-platelet therapy based upon platelet function testing**

Prospective, albeit small, studies have provided evidence that HPR is not just a diagnostic marker but a modifiable risk factor with tailored anti-platelet therapy. In two prospective trials, tailored incremental loading doses of clopidogrel before PCI overcame HPR and were effective in reducing major 30-day adverse cardiac events (58, 59). Similarly, two other studies demonstrated that selective GPIIb/IIIa receptor blocker administration administered to PCI patients with HPR following clopidogrel loading was effective in reducing subsequent post-PCI short- (periprocedural) as well as long-term (one year) ischaemic outcomes (60, 61). These studies were the first to suggest that the cut-off value used to identify PCI-treated patients at increased risk of thrombotic event occurrence was also useful to tailor therapy and lead to an improved outcome.

In the GRAVITAS trial, the first large scale investigation of personalised antiplatelet therapy in the elective PCI patient with HPR, high-dose clopidogrel treatment (150 mg/day) resulted in the same six-month composite ischaemic event occurrence as standard dose treatment (75 mg/day) (34). A potential explanation was that high-dose clopidogrel was a suboptimal remedy to overcome HPR; indeed, the prevalence of HPR at 30 days was 40% in patients treated with high maintenance dose clopidogrel. Furthermore, only a highly effective remedy to reduce HPR could produce a clinically measurable difference in outcome in a study with an event rate as low as that observed in GRAVITAS (2.3%). Finally, the cut-off (>230 PRU) for HPR (i.e. threshold at which patients were included in the trial) may have been too high (62). In a time-coxivariate Cox regression analysis of on-treatment platelet reactivity in GRAVITAS, a PRU <208 was an independent predictor of event-free survival at 60 days (hazard ratio [HR] 0.23, p=0.047) with a consistent trend also evident at six months (HR 0.54, p=0.06) (63). In the recently presented Testing platelet Reactivity In patients undergoing elective stent placement on clopidogrel to Guide alternative therapy with prasugrel (TRIGER-PCI) study, a 10 mg daily dose of prasugrel was effective in reducing on-treatment platelet reactivity compared to 75 mg daily dose clopidogrel (64). The latter personalised antiplatelet trial used >208 PRU a cut-off point for HPR. However, the study was terminated early for futility because of extremely low event rates.

**Potential optimisation of the HPR cut-off**

At this time many important issues remain unresolved. The HPR threshold mentioned in the consensus statement was determined by ROC analysis and is only applicable to the PCI population. However, based on the group of patients from GRAVITAS treated with standard-dose clopidogrel, an even lower threshold defining HPR (∼170 PRU) was associated with optimal identification of patients destined to experience ischaemic event occurrence. It was suggested that this “immunity to thrombosis” cut-off should be considered as the new therapeutic target in the PCI patient (65). During the early phase of ACS and/or PCI, disease activity is greatest, and the prevalence of clopidogrel non-responsiveness level is higher. At that time a potent antiplatelet regimen may provide the greatest net clinical benefit (reduction in ischaemic events that outweighs the risk of bleeding events) whereas at time points further downstream from the ACS event, less intense antiplatelet effects may be desirable. The optimal HPR threshold at ∼30 days may therefore differ from the acute threshold during the index ACS hospitalisation. The HPR cut-offs proposed thus far are based on a single measurement [either before PCI (mostly European studies) or before discharge (American studies)]. Longitudinal platelet function measurements have never been done in a large scale antiplatelet therapy trial in ACS patients. Knowledge gained from such an analysis will enhance our understanding of the relationship between platelet reactivity, and ischaemic and bleeding event occurrences.

It should be taken into consideration that the currently accepted HPR cut-off values have been associated in many studies with modestly increased odds ratios for ischaemic event occurrence and are associated with high negative predictive values (NPVs) and low positive predictive values (PPVs). However, given the overall low prevalence of thrombotic events in these studies, the low PPV is understandable. Moreover, there is debate about whether diagnostic test statistics were appropriately used to describe the utility of prognostic tests, such as platelet function tests. The current data indicate that although platelet reactivity plays a major role in ischaemic event occurrence (up to 50% of the attributable risk of 30-day stent thrombosis in ADEPT-DES), other factors including demographic and clinical factors must be taken into consideration to optimally define the patients at greatest risk. Along this line, recent studies also suggest that adding clinical variables and genotype to platelet reactivity measurements (combined risk factor) will improve risk prediction (66, 67).

Thus far, the relation of platelet reactivity to bleeding has not been investigated in a systematic fashion within a large-scale prospective clinical trial. The ability to evaluate bleeding susceptibility is highly relevant, as it may enable the tailoring of antiplatelet therapy to enhance the risk-benefit balance and net clinical benefit during DAPT particularly with potent P2Y_{12} receptor blocker therapy.

There have been no randomised prospective trials focused exclusively on the use of DAPT in medically managed ACS patients. Antiplatelet therapy monitoring to study the relation of HPR and genotype to clinical outcome in these patients is completely unknown. Moreover, the stability of the HPR phenotype in medically managed ACS patients is very poorly understood as there is no information in patients managed without PCI. Finally, the relation of platelet reactivity and genotype to bleeding risk has been much less studied as compared to the relation to ischaemic event occurrence.
Asian populations have a two-fold greater prevalence of CYP2C19 LoF alleles and much lower prevalence of GOF alleles compared with Caucasian populations, therefore, high on-treatment platelet reactivity can potentially impact post-ACS outcomes to a greater extent in Asian populations (68). Data on HPR thresholds predicting ischaemic and bleeding outcomes remain poorly defined in Asians.

Patients with type 2 diabetes mellitus have a reduced response to clopidogrel and have a higher risk of ischaemic events as compared with patients who do not have diabetes (69, 70). In the TRITON-TIMI 38 trial, the absolute improvement in clinical outcomes with prasugrel, compared with clopidogrel, was greater in patients with diabetes than those without diabetes and this efficacy was not offset by increased bleeding (23). Further studies are needed to clearly establish the relation between platelet function and clinical outcomes in patients with diabetes.

### The TRILOGY-ACS platelet function Substudy

The TRILOGY-ACS (TaRgeted platelet Inhibition to cLarify the Optimal strateGy to medically manage Acute Coronary Syndromes) study is a global phase III, double-blind, double-dummy, parallel-group randomised-controlled trial comparing prasugrel with clopidogrel among medically-managed non-ST elevation (NSTE) ACS patients. The study will enroll approximately 10,300 subjects at 800 sites globally (7,800 subjects <75 years of age and ≥75 years of age) (71). Approximately one third of the TRILOGY-ACS study population is expected to be enrolled in the platelet function substudy where platelet function will be assessed with the VerifyNow® P2Y12 and Aspirin assays at multiple time-points throughout the study including before and after switching from clopidogrel to prasugrel (►Tables 4 and 5) (71). In addition, extensive analyses of SNPs as-

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| **Important studies linking platelet function measurement to bleeding.** | | | |

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Table 4: Study drug treatment by commercial clopidogrel status in the TRILOGY-ACS substudy. *Loading dose defined as at least 300 mg of commercial clopidogrel. †Steady state defined as having received maintenance dose of commercial clopidogrel for at least five consecutive days immediately prior to the index event. ‡Subjects aged ≥75 years or with body weight <60 kg will receive 5-mg maintenance dose. Adapted with permission from (56). On day 1, 4 blood samples will be obtained from subjects enrolled in the platelet function substudy—pre-dose P2Y12 assay, post-dose P2Y12 assay, aspirin assay and blood for pharmacogenomic analysis. During visit 3, two blood samples will be drawn, and for all other visits, only one blood sample will be drawn.

<table>
<thead>
<tr>
<th>Stratum</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stratum 1: Not on a stable regimen of clopidogrel (either does not have commercial clopidogrel loading dose* or is not at steady state† on commercial clopidogrel) and randomisation within 72 h following onset of index event</td>
<td>Loading dose/maintenance dose: Clopidogrel 300 mg loading dose followed by 75 mg/day maintenance dose; or Prasugrel 30 mg loading dose followed by 5† or 10 mg/day maintenance dose</td>
</tr>
<tr>
<td>Stratum 2: Not on a stable regimen of clopidogrel: Commercial clopidogrel loading dose* initiated within 72 h following onset of index event with administration of daily maintenance dose until randomisation</td>
<td>Maintenance dose only: Clopidogrel 75 mg/day or prasugrel 5† or 10 mg/day</td>
</tr>
<tr>
<td>Stratum 3: On a stable regimen of clopidogrel prior to index event (at steady state† on commercial clopidogrel) with administration of daily maintenance dose until randomisation</td>
<td>Maintenance dose only: Clopidogrel 75 mg/day or prasugrel 5† or 10 mg/day</td>
</tr>
</tbody>
</table>

Table 5: Schedule of platelet function measurement in the TRILOGY-ACS substudy.  
† Pre-dose P2Y12 assay before 1st dose of study drug, and post-dose P2Y12 assay 2 hours after 1st dose of study drug.

Conclusions

There are many gaps in our knowledge regarding the role of platelet function and genetic testing to optimise anti-platelet therapy including 1) no information on stable coronary disease patients, 2) no information on the relation of phenotype to events in medically managed ACS patients, 3) few data on the relation of long term platelet reactivity to both ischaemic and bleeding events, 4) preliminary data only on the relation of phenotype and genotype to bleeding, 5) limited data on the utility of combining genotype and phenotype data for prognosis, 6) uncertainty regarding the variability of platelet function over time, 7) limited data relating platelet function to clinical outcomes in a major clinical trial of antiplatelet therapy, and most importantly, 8) limited evidence from a large-scale trial that personalisation of antiplatelet therapy enhances efficacy and improves safety. Ongoing studies, including the TRILOGY ACS Platelet Function Substudy, and future studies, will provide valuable information and potentially influence the field of personalised antiplatelet therapy.

Conflicts of interest

Dr. Gurbel has received research grant/consultation fees/honoraria from Pozen, AstraZeneca, Novartis, Bayer, Eli Lilly, Daiichi Sankyo, Accuremetrics, Sanofi-Aventis, Merck, Medtronic, and Boehringer Ingelheim. Dr. Matthew Roe has received research funding from Eli Lilly, Roche, Bristol-Myers Squibb, American College of Cardiology, American Heart Association, and consulting fees or honoraria from KAI Pharmaceuticals, Bristol-Myers Squibb, Sanofi-Aventis, Merck, Orexigen, Helsinn Pharmaceuticals, Astra Zeneca, and Regeneron. Dr. Ohman has received research grants/consulting fees from AstraZeneca, Boehringer Ingelheim, Bristol Meyers Squibb, Daiichi Sankyo, Eli Lilly, Gilead Sciences, Liposcience, Maquet, Merck, Pozen, Roche, Sanofi-Aventis, The Medicines Company, and WebMD. Dr. Tantry has received honoraria from Bayer and Accuremetrics. Dr. Jakubowski is an employee of Eli Lilly and Company. Dr. Chan has received grant and consulting fees from Eli-Lilly and Astraeneca. Dr. Cor-

Visit no. | 1 (Day 1) | 3 (Day 30) | 4, 5, 7, 9, 11, and 13 (Months 3, 6, 12, 18, 24, and 30), early discontinuation and final discontinuation
---|---|---|---
Tests required | Pre-dose P2Y12 assay, post-dose P2Y12 assay, aspirin assay and pharmacogenomic sample | Post-dose P2Y12 assay, and aspirin assay | Post-dose P2Y12 assay
nel has received advisory board and consulting fees from AstraZeneca and Eli-Lilly/Daiichi Sankyo. Dr. Goodman has received research grant/consultation fees/honoraria from AstraZeneca, Novartis, Bayer, Eli Lilly, Daiichi Sankyo, Sanofi-Aventis, Merck, Eisai, The Medicine Co. None of the other authors declares any conflicts of interest.

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