Intrinsic platelet reactivity before P2Y\textsubscript{12} blockade contributes to residual platelet reactivity despite high-level P2Y\textsubscript{12} blockade by prasugrel or high-dose clopidogrel

Results from PRINCIPLE-TIMI 44

Andrew L. Frelinger III\textsuperscript{1,2}; Alan D. Michelson\textsuperscript{1,2}; Stephen D. Wiviott\textsuperscript{3}; Dietmar Trenk\textsuperscript{4}; Franz-Josef Neumann\textsuperscript{4}; Debra L. Miller\textsuperscript{5}; Joseph A. Jakubowski-Ti\textsuperscript{5}; Timothy M. Costigan\textsuperscript{3}; Carolyn H. McCabe\textsuperscript{3}; Elliott M. Antman\textsuperscript{3}; Eugene Braunwald\textsuperscript{3}

\textsuperscript{1}Center for Platelet Research Studies, Division of Hematology/Oncology, Children’s Hospital Boston, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA; \textsuperscript{2}Center for Platelet Function Studies, Department of Pediatrics, University of Massachusetts Medical School, Worcester, Massachusetts, USA; \textsuperscript{3}TIMI Study Group, Cardiovascular Division, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts, USA; \textsuperscript{4}Herz-Zentrum Bad Krozingen, Bad Krozingen, Germany; \textsuperscript{5}Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, Indiana, USA

Summary

It was the objective of this study to determine whether the intrinsic platelet response to adenosine diphosphate (ADP) before thienopyridine exposure contributes to residual platelet reactivity to ADP despite high level P2Y\textsubscript{12} blockade by prasugrel (60 mg loading dose [LD]), 10 mg daily maintenance dose [MD]) or high-dose clopidogrel (600 mg LD, 150 mg daily MD). High residual platelet function during clopidogrel therapy is associated with poor clinical outcomes. It remains unknown whether the relationship between platelet reactivity prior to treatment with clopidogrel (300 mg LD, 75 mg daily MD) and residual on-treatment platelet reactivity is maintained after more potent P2Y\textsubscript{12} inhibition. PRINCIPLE-TIMI 44 was a randomised, double-blind, two-phase crossover study of prasugrel compared with high-dose clopidogrel in 201 patients undergoing cardiac catheterisation for planned percutaneous coronary intervention. ADP-stimulated platelet-monocyte aggregates, platelet surface P-selectin and platelet aggregation were measured pre-treatment, during LD (6 h and 18–24 h) and MD (15 d).

Correlations of pre-treatment to on-treatment values were determined by Spearman rank order. Prasugrel resulted in greater platelet inhibition than high-dose clopidogrel for each measure. However, for both drugs, pre-treatment reactivity to ADP predicted 6 h, 18–24 h and 15 day reactivity to ADP (correlations 0.24–0.62 for platelet-monocyte aggregates and P-selectin). In conclusion, a patient’s intrinsic platelet response to ADP before exposure to thienopyridines contributes to residual platelet reactivity to ADP despite high level P2Y\textsubscript{12} blockade with high-dose clopidogrel or even higher level P2Y\textsubscript{12} blockade with prasugrel. Patients who are hyper-responsive to ADP pre-treatment are more likely to be hyper-responsive to ADP on-treatment, which may be relevant to therapeutic strategies.

Keywords

Antiplatelet agents, cardiology, clinical trials, antiplatelet drugs, platelet pharmacology

Introduction

Patient-to-patient variability in residual platelet function during clopidogrel therapy has been well described (1). Multiple studies indicate high residual platelet reactivity, also referred to as clopidogrel “resistance” or “non-responsiveness”, is associated with poor clinical outcomes (1–5). However, we (6) and others (2) have suggested that a portion of platelet function following treatment with standard dose clopidogrel (300 mg loading dose [LD], 75 mg daily maintenance dose [MD]) is accounted for by pretreatment platelet reactivity – and that clopidogrel “resistance” or “non-responsiveness” may therefore not be appropriate terms.

More potent P2Y\textsubscript{12} antagonism is now being used more frequently in patients, either high-dose clopidogrel (600 mg LD, 150 mg daily MD) or the recently Food and Drug Administration (FDA)-approved prasugrel (7–9). However, it remains unknown whether the relationship between platelet reactivity prior to exposure to thienopyridines and residual on-treatment platelet reactivity (2, 6) is maintained in patients after this more potent P2Y\textsubscript{12} inhibition – or whether more potent P2Y\textsubscript{12} antagonism can over-
come the influence of the pretreatment platelet reactivity. The present study examined this question in PRINCIPLE-TIMI 44, a randomised, double-blind, double-dummy study of prasugrel compared with high-dose clopidogrel in 201 patients undergoing cardiac catheterisation for planned percutaneous coronary intervention (PCI) (9).

Materials and methods

Study protocol

PRINCIPLE-TIMI 44 was a randomised, double-blind, double-dummy, active comparator-controlled, two-phase crossover study of prasugrel versus high LD and MD clopidogrel in patients undergoing cardiac catheterisation for planned PCI (9). Patients (n = 201) were recruited from 14 sites in four countries. The protocol was approved by the institutional review boards at all participating centres and the patients gave informed consent. Patients were randomised to treatment with prasugrel 60 mg or clopidogrel 600 mg LD. After randomisation and before receiving study drug, all patients were to have blood obtained for baseline platelet measures, including adenosine diphosphate (ADP)-stimulated platelet-monocyte aggregates, platelet surface P-selectin and light transmission aggregation (LTA). Blood was drawn from venipuncture or indwelling catheters. The first 5 ml were to be discarded. The LD of the blinded study drug was given as a pretreatment ~1 hour (h) (no less than 30 minutes [min]) before the time that cardiac catheterisation was expected to begin. Patients were then to undergo PCI if coronary anatomy was suitable. After PCI, patients were to receive a once-daily MD for 14 ± 2 days of either prasugrel 10 mg or clopidogrel 150 mg corresponding to the LD assignment. A 15 ± 2-day visit was performed for collection of end points, safety information, and platelet measures with the patients crossing over directly to the alternate maintenance therapy for an additional 14 ± 2 days without an intervening washout period. A 29 ± 4-day follow-up visit was performed with assessment of clinical endpoints, safety information, and platelet measures (9).

This was an intent-to-treat study. The LD-phase analysis population consisted of patients who received the LD of the study drug and did not receive a GP IIb/IIIa inhibitor. This population was used for all analyses of platelet function measures within 24 h after the LD, although only patients with PCI had 18– to 24-h measures. The MD-phase analysis population consisted of patients who received a PCI regardless of whether they received a GP IIb/IIIa inhibitor. This population was used for all analyses of platelet function measures 24 h after the LD. In every instance, the analyses considered only patients with evaluable measurements available for a given time point.

All patients received aspirin, although 13% were aspirin-naive at baseline (pre-treatment). In subjects not receiving chronic aspirin therapy, oral enteric-coated aspirin 325 to 500 mg was administered with the loading dose of the study drug. A daily enteric-coated aspirin (75 to 325 mg) was administered to each subject during the study.

Platelet function tests

ADP 20 μM stimulated platelet-monocyte aggregates and platelet surface P-selectin were measured by flow cytometry pre-treatment, during loading (6 h and 18–24 h) and maintenance phase (15 days). To minimise site-to-site variation of these assays in this multi-site international study, measures were taken to standardise reagents, core instrumentation, and training of site personnel. Specifically, for all sites worldwide, the Center for Platelet Function Studies at the University of Massachusetts Medical School provided ADP and buffer solutions for the platelet-monocyte aggregates and platelet surface P-selectin assays as pre-measured, single-use, frozen aliquots. Flow cytometry was performed at two core facilities: the Center for Platelet Function Studies, University of Massachusetts Medical School, Worcester, for U.S. sites; and Herz-Zentrum, Bad Krozingen, Germany, for European and Israeli sites. Both flow cytometry core facilities used Becton Dickinson FACSCalibur™ instruments (Becton Dickinson, Franklin Lakes, NJ, USA) calibrated daily to matching specifications using a single lot of Spheroid Rainbow Calibration Particles (Spherotech Inc., Libertyville, IL, USA). Both instruments were run using CellQuest Pro software (Becton Dickinson) and both flow cytometry core facilities used the same standardised protocols for processing and analysing samples. Training sessions conducted by the Center for Platelet Function Studies for laboratory personnel were held in Worcester, MA, USA, Frankfurt and Bad Krozingen, Germany, and Tel Aviv, Israel.

Blood for the platelet-monocyte aggregate and platelet surface P-selectin assays was drawn into 3.2% citrate anticoagulant. For the platelet-monocyte aggregate assay, 100 μl of blood was added within 15 min of collection to tubes containing a) 100 μl 10 mM Hepes-Tyrode’s buffer and b) 100 μl 40 μl ADP in 10 mM Hepes-Tyrode’s buffer (kept frozen until use). After 15 min incubation at room temperature, 1 ml of FACS Lysing Solution (Becton Dickinson) was added and the samples were refrigerated until shipping by overnight courier to a flow cytometry core facility. For the platelet surface P-selectin assay, 10 μl of citrated blood was added to tubes containing a) 90 μl 10 mM Hepes-Tyrode’s buffer and b) 90 μl 22.2 μM ADP in 10 mM Hepes-Tyrode’s buffer (supplied by the Center for Platelet Function Studies and kept frozen until use). After 15 min incubation at room temperature, 100 μl of 2% formaldehyde (Polysciences, Warrington, PA, USA) was added and the samples refrigerated until shipping to a flow cytometry core facility. At the flow cytometry core facilities, platelet-monocyte aggregate samples were centrifuged (280 x g for 5 min), and the resulting cell pellet was resuspended in 200 μl 10 mM HEPES, 0.15 M NaCl, 0.5% bovine serum albumin, pH 7.4. Resuspended cells (80 μl) were added to tubes containing 20 μl of phycoerythrin-cyanin 5 (PC5) conjugated anti-CD14 (to identify monocytes, Beckman Coulter, Fullerton, CA, USA) and either phycoerythrin (PE)-conjugated mouse IgG1 isotype control antibody or PE-CD42a monoclonal antibody (clone ALMA-16 directed against GPiX) for platelet identification (BD Pharmingen, San Diego, CA, USA). After 40 min incubation at room temperature, 600 μl of 1% formaldehyde in 10 mM HEPES, 0.15 M NaCl was added and the samples stored at 4°C until analysis.
Flow cytometric analysis of platelet-monocyte aggregates was performed essentially as previously described (10) with the threshold set on forward light scatter to eliminate cells smaller than leukocytes, and using a combination of CD14 staining and side light scatter to positively identify the monocyte population. The % platelet-monocyte aggregates, i.e. the % of monocytes with platelet-specific marker fluorescence (PE-CD42a) above background levels (set using the isotype control antibody), was then evaluated.

Platelet surface P-selectin samples were processed at the flow cytometry core facilities by adding 20 μl of sample to tubes containing 20 μl of fluorescein-conjugated CD61 (for platelet identification, clone Y2/51, DAKO, Carpinteria, CA, USA) with either PE-conjugated mouse IgG2a or PE-conjugated CD62P (both from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). After 40 min incubation at room temperature, 600 μl of 1% formaldehyde in 10 mM HEPES, 0.15 M NaCl was added and the samples stored at 4°C until analysis. Flow cytometric analysis of platelet surface P-selectin was performed essentially as previously described (10). CD61 fluorescence and characteristic forward and side light scatter was used to identify platelets. A positive analysis region was set so that only 1% of cells stained with the isotype control antibody were positive.

ADP-induced LTA was performed as previously described (9). Before study participation, study participants were trained by core laboratory personnel to ensure consistent LTA protocols among sites, and local tracings were reviewed for quality before the first patient was randomised at each site. For LTA, blood was drawn into a 4.5 ml 3.2% citrate Vacutainer tube and centrifuged to prepare platelet-rich plasma (PRP) and platelet-poor plasma (PPP). ADP aliquots were prepared centrally and shipped to investigative sites. LTA was determined with 5 μM and 20 μM ADP separately. Maximal % platelet aggregation was determined by blinded laboratory personnel. Evidence of haemolysis, platelet count of the PRP, and procedural irregularities were reported. All tracings were read locally and overread by a single reader blinded to treatment assignment at a central core laboratory (Center for Platelet Function Studies, University of Massachusetts, Worcester). Central readings were used for all analyses.

**Statistical methods**

An ANCOVA model with factors for study site, core laboratory, baseline value, and study treatment was used to compare platelet function in prasugrel- versus clopidogrel-treated patients at each of the indicated on-treatment times. Spearman rank order correlation was used to analyse non-Gaussian distributed results of
pre-thienopyridine platelet reactivity and on-treatment platelet reactivity. Pearson correlations were used following transformation of non-Gaussian results, using the equation $X_{\text{transformed}} = (100 - X_{\text{raw}})^{0.5}$, where $X$ is the percent platelet-monocyte aggregates or platelet surface P-selectin.

**Results**

Patient enrolment and patient demographics have been previously described (9). Briefly, patients were more frequently male, had a mean age of 63.9 years (range 56–71), body mass index (BMI) 29.0 kg/m², 29.4% had a history of prior myocardial infarction, 19.4% had prior coronary artery bypass grafting surgery, 30.8% had diabetes mellitus, 16.9% were current smokers, 87.4% were on aspirin, 80.1% were on a beta blocker, 89.5% were on a statin, and 55.8% had PCI for an index event. Baseline features of the randomised patients were well matched (Table 1 of reference [9]).

The flow of patients through the trial and the number evaluable for the individual endpoints are shown in Figure 1. There were 201 patients randomised at 14 investigative sites in four countries. Four patients received a GP IIb/IIIa inhibitor at the time of PCI and were excluded from LD-phase analyses, which therefore included 197 patients (99 prasugrel, 98 clopidogrel). There were no statistically significant differences in the number of evaluable patients by treatment (clopidogrel vs. prasugrel) or differences in baseline characteristics of evaluable compared with non-evaluable patients or between non-evaluable patients compared by treatment group.

Irrespective of pre-treatment platelet function, prasugrel 60 mg LD and 10 mg daily MD resulted in greater platelet inhibition than high-dose clopidogrel (600 mg LD and 150 mg MD), as determined by lower percentages of 20 μM ADP-induced platelet-monocyte aggregates (Fig. 2A) and platelet surface P-selectin (Fig. 2B) in both the loading phase (up to 24 h) and the maintenance phase (15 days). These findings in patients with high level P2Y12 blockade undergoing cardiac catheterisation for planned PCI extend previous findings in patients with stable coronary artery disease on a standard 75 mg daily MD of clopidogrel (11). We previously reported similar results for 5 μM and 20 μM ADP-induced LTA (9).

Preliminary evaluation of the distribution of platelet function results pre-thienopyridine and on-treatment demonstrated non-Gaussian distributions. Therefore, to evaluate whether pre-existent platelet function prior to thienopyridine treatment predicts on-treatment platelet function, correlations of pre-treatment to on-treatment values were determined by Spearman rank order (one-sided, $p < 0.05$ considered significant) (Figs. 3 and 4).

For patients treated with either prasugrel or high-dose clopidogrel, pre-treatment reactivity to ADP, as determined by platelet-monocyte aggregates, predicted 6 h, 18–24 h and 15 day reactivity to ADP (correlations 0.30–0.62, $p < 0.0001 – 0.0156$) (Fig. 3A, C, and E). Likewise, for patients treated with either prasugrel or high-dose clopidogrel, pre-treatment reactivity to ADP, as determined by platelet-monocyte aggregates, predicted 6 h, 18–24 h and 15 day reactivity to ADP (correlations 0.24–0.59, $p < 0.001 – 0.0455$) (Fig. 3B, D, and F). Statistically significant Pearson correlation coefficients ($p < 0.0001 – 0.049$) were also obtained after normalisation of the data to produce a Gaussian distribution by using the equation $X = (100 - X)^{0.5}$, where $X$ is the percent platelet-monocyte aggregates or platelet surface P-selectin (data not shown). For patients treated with either prasugrel or high-dose clopidogrel, pre-treatment reactivity to 5 μM ADP, as determined by LTA, predicted 6 h reactivity to ADP.
ADP (correlation 0.33, p = 0.0012–0.0013) (Fig. 4A) but this effect was blunted at the higher concentration of ADP (20 μM) in this assay (Fig. 4B) because of the narrower range of pre-treatment responses. Because LTA results are often reported as the % inhibition of platelet aggregation (IPA) relative to baseline, we also determined the correlation of on-treatment IPA with pre-treatment platelet reactivity (as measured by maximal % platelet aggregation, since by definition pre-treatment IPA is 0%). Prasugrel resulted in greater IPA than high-dose clopidogrel and this inhibition was not correlated with pre-treatment platelet reactivity (Fig. 4C, D, G, H, K, and L). In contrast, high-dose clopidogrel resulted in lower IPA, which at 18–24 h and 15 days was positively correlated with pre-treatment platelet reactivity (correlations 0.29–0.41, p 0.0023–0.0283) (Fig. 4G, H, K, and L).

**Discussion**

No previous studies have examined the relationship between intrinsic platelet response to ADP before thienopyridine exposure and residual platelet reactivity to ADP in the setting of high-level P2Y₁₂ blockade by prasugrel or high-dose clopidogrel. In this randomised, double-blind, double-dummy trial of prasugrel (60 mg LD, 10 mg daily MD) versus high-dose clopidogrel (600 mg LD, 150 mg daily MD) in patients undergoing cardiac catheterisation for planned PCI, we have demonstrated that the intrinsic platelet response to ADP before P2Y₁₂ blockade contributes to the residual platelet reactivity despite high-level P2Y₁₂ blockade with high-dose clopidogrel or even higher level P2Y₁₂ blockade with prasugrel. These findings were reproducible with different measures of platelet reactivity (platelet-monocyte aggregates, platelet surface P-selectin and, to a lesser extent, with LTA) and different time points after the institution of thienopyridine treatment (6 h, 18–24 h, and 15 days on-treatment). Platelet-monocyte aggregates and platelet surface P-selectin are important measures be-
Figure 4: Correlation of platelet reactivity (ADP-stimulated light transmission aggregation [LTA] and inhibition of platelet aggregation [IPA]) after prasugrel or high-dose clopidogrel with pre-thienopyridine platelet reactivity. A, E, and I) Pre-thienopyridine vs. on-treatment 5 μM ADP-stimulated LTA. B, F, and J) Pre-thienopyridine vs. on-treatment 20 μM ADP-stimulated LTA. C, G, and K) Pre-thienopyridine 5 μM ADP-stimulated LTA vs. on-treatment IPA with 5 μM ADP. D, H, and L) Pre-thienopyridine 20 μM ADP-stimulated LTA vs. on-treatment IPA with 20 μM ADP. A – D) 6 h on-treatment with thienopyridine. E – H) 18–24 h on-treatment with thienopyridine. I – L) 15 days on-treatment with thienopyridine. Clopidogrel: open circles, dashed lines. Prasugrel: closed circles, solid lines. r_s, Spearman rank order correlation coefficient.
cause they contribute to tissue factor generation and have pro-
coagulant consequences (12).

The lower correlations of pre-thienopyridine treatment versus
on-treatment platelet reactivity measured by LTA (Fig. 4A, B, E,
F, I, J) than by platelet-monocyte aggregates or platelet surface
P-selectin (Fig. 3) are not unexpected because, although LTA has
been “grandfathered in” as the quasi-gold standard measurement
of platelet function (13), it is semiquantitative and measures an
event well downstream from P2Y_{12} blockade – making it depend-
ent on many other factors (e.g. GPIIb-IIIa receptor density, fibrin-
ogen concentration). Pre-treatment platelet reactivity measured
by LTA correlated with IPA in patients treated with high-dose
clopidogrel (Fig. 4G, H, K, L). Patients with higher pre-treat-
ment maximal platelet aggregation treated with high-dose clopi-
dogrel had higher IPAs than patients with lower pre-treatment
maximal platelet aggregation treated with high-dose clopidogrel.
In contrast, patients treated with prasugrel had a consistent, high
level of IPA regardless of the pre-treatment level of platelet reac-
tivity measured by LTA (Fig. 4C, D, G, H, K, L). While IPA is clearly
important, there is a growing consensus that residual high on-
treatment platelet reactivity as reflected by maximal platelet aggre-
gation with 5 μM ADP may be a more useful predictor of throm-
botic risk (14).

We and others have previously suggested that a portion of platelet
function following treatment with standard-dose clopidogrel (300
mg LD, 75 mg daily MD) is accounted for by pretreatment platelet
reactivity (2, 6) – and that clopidogrel “resistance” may therefore not
be an appropriate term. Nevertheless the terms “clopidogrel resis-
tance” and “clopidogrel nonresponsiveness” continue to be widely
used (1, 15–20). These terms implicitly assume that all on-treatment
platelet reactivity is dependent on the effects of clopidogrel. How-
ever, the present randomised, double-blind, double-dummy study
demonstrates that the terms “clopidogrel resistance” and “clopi-
dogrel nonresponsiveness” are inappropriate – because the residual
platelet reactivity after clopidogrel treatment is in part unrelated
to clopidogrel but is dependent on the pre-clopidogrel intrinsic vari-
ability in platelet activation. A preferred term is “high on-treatment
platelet reactivity” (14, 21, 22). Indeed, a recent JACC white paper
(14) concluded that the absolute level of platelet reactivity during
clopidogrel treatment (i.e. on-treatment platelet reactivity) is a
better measure of thrombotic risk than platelet responsiveness
to clopidogrel. This conclusion implies that pre-clopidogrel platelet
responsiveness is an important factor in thrombotic risk, and is con-
sistent with the findings of the present study that a patient’s intrin-
sis platelet response to ADP before exposure to thienopyridines con-
tributes to on-treatment platelet reactivity to ADP.

The underlying mechanisms for the pre-clopidogrel, inter-indi-
vidual variability in ADP-induced platelet reactivity are still in-
completely understood but have been reported to include single
nucleotide polymorphisms (SNPs) in the genes for the P2Y_{12} and/or
P2Y_{13} ADP receptors (23, 24) and/or variations in the platelet
surface density of the P2Y_{12} receptor (25). Recently, Jones et al. (26),
used a functional genomics approach to compare a relatively large
number of SNPs (n = 1,327) in a selected group of candidate genes
(n = 97), resulting in the identification of 17 independently associ-
ated SNPs that account for 48% of the variation in platelet reactiv-
ity induced by ADP. Even more recently, Johnson et al. (27) per-
formed a genome-wide meta-analysis of 2.5 million SNPs in 4,831
subjects to identify three loci associated with ADP-induced platelet
aggregation.

The widely studied CYP2C19*2 genotype accounts for only ap-
proximately 12% of the variability in clopidogrel response (28). In
contrast, our data show that one can predict, with correlations be-
 tween 0.24 and 0.62 (depending on the assay and time point – see
Figs. 3 and 4), which individuals will or will not achieve a prede-
fined target level of platelet inhibition (although the range of resid-
ual platelet reactivity is significantly lower in prasugrel-treated pa-
tients). For example, a patient with 90% ADP-stimulated platelet-
monocyte aggregation pre-thienopyridine treatment would be pre-
dicted to have 32% ADP-stimulated platelet-monocyte aggrega-
tion 6 h after prasugrel treatment or 70% ADP-stimulated pla-
et-monocyte aggregation 6 h after high-dose clopidogrel treat-
ment. Similarly, a patient with 75% ADP-stimulated platelet-
monocyte aggregation pre-thienopyridine treatment would be pre-
dicted to have 27% ADP-stimulated platelet-monocyte aggrega-
tion 6 h after prasugrel treatment or 60% ADP-stimulated pla-
et-monocyte aggregation 6 h after high-dose clopidogrel treat-
ment. Thus, a hypothetical target level of ≤60% ADP-stimulated
platelet-monocyte aggregates would be predicted to be achieved in
the first example with prasugrel but not with high-dose clopi-
dogrel, whereas in the latter example ≤60% ADP-stimulated platelet-
monocyte aggregates would be predicted to be achieved with either
therapy. However, given the very wide confidence intervals for such
predictions, on-treatment testing may provide more useful infor-
mation if clinical outcomes studies identify a target therapeutic
level of platelet function (14). Nevertheless, our findings raise the
possibility that: i) patients whose platelets are more reactive to
ADP prior to thienopyridine treatment may be at greater risk for
thrombosis, and may therefore preferentially benefit from more
potent antiplatelet therapy; ii) patients whose platelets are less re-
sponsive to ADP prior to thienopyridine treatment may be at
greater risk for haemorrhage, and may therefore preferentially
benefit from less potent antiplatelet therapy. However, these hypo-
theses would need to be prospectively tested in a clinical trial.

Limitations

Platelet function testing requires very precise sample conditions
and processing. Even among expert sites with experience in platelet
function testing, some samples did not meet pre-specified con-
ditions and were excluded from analyses (Fig. 1). However, as re-
ported in the original PRINCIPLE TIMI-44 manuscript (9), there
were no significant differences in baseline characteristics among
patients who were evaluable or non-evaluable for the primary end-
points or among non-evaluable patients between therapies, mak-
ing any influence on the study outcomes unlikely. Phosphorylation
of vasodilator-stimulated phosphoprotein (VASP), although
measured in PRINCIPLE-TIMI 44 (9), was not studied in this

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Fig. 4A, B, E,
analysis because of the uniformity of pre-treatment response to ADP (88.2 ± 6.4 platelet reactivity units [PRI], mean ± SD, n = 131) – rendering this test uninformative as to whether pre-treatment response predicts on-treatment response.

Conflict of interests

Dr. Frelinger: research grants from GLSynthesis, Lilly/Daiichi Sankyo and Sanofi Aventis/Bristol-Myers Squibb; consultant to Eli Lilly. Dr. Michelson: research grants from Lilly/Daiichi Sankyo and Sanofi Aventis/Bristol-Myers Squibb; member of the Data Safety Monitoring Board of Clopidogrel to Lower Arterial Thrombotic Risk in Neonates and Infants Trial (CLARINET) (a clinical trial sponsored by Sanofi Aventis/Bristol-Myers Squibb); consultant to Lilly/Daiichi Sankyo. Dr. Wiviott: research grants from Lilly/Daiichi Sankyo and Schering Plough; CME honoraria from Astra Zeneca, Lilly/Daiichi Sankyo and Schering Plough; consultant to AstraZeneca and Bristol-Myers Squibb/Sanofi Aventis. Dr. Trenk: research grant from Eli Lilly; speakers’ bureau for Lilly/Daiichi Sankyo; consultant to Astra Zeneca and Lilly/Daiichi Sankyo. Ms. Miller and Drs. Jakubowski and Costigan are employees of Eli Lilly, Dr. Antman: research grants from Astra Zeneca, Lilly/Daiichi Sankyo and Sanofi Aventis/Bristol-Myers Squibb. Dr. Braunwald: research grants from Lilly/Daiichi Sankyo; honoraria from Eli Lilly; Daiichi Sankyo advisory board; consultant to worldwide clinical trials. Dr. Neumann and Ms. McCabe report no disclosures.

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