
Article:
Storey, R.F. orcid.org/0000-0002-6677-6229, Angiolillo, D.J., Bonaca, M.P. et al. (12 more authors) (2016) Platelet inhibition with ticagrelor 60 mg versus 90 mg twice daily in the PEGASUS-TIMI 54 trial. Journal of the American College of Cardiology, 67 (10). pp. 1145-1154. ISSN 0735-1097

https://doi.org/10.1016/j.jacc.2015.12.062

JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY VOL. 67, NO. 10, 2016
ISSN 0735-1097
http://dx.doi.org/10.1016/j.jacc.2015.12.062

Platelet Inhibition With Ticagrelor 60 mg Versus 90 mg Twice Daily in the PEGASUS-TIMI 54 Trial

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ABSTRACT

BACKGROUND The PEGASUS-TIMI 54 (Prevention of Cardiovascular Events in Patients with Prior Heart Attack Using Ticagrelor Compared to Placebo on a Background of Aspirin–Thrombolysis In Myocardial Infarction 54) trial studied 2 doses of ticagrelor, 90 mg twice a day (bid) and 60 mg bid, for long-term prevention of ischemic events in patients with prior myocardial infarction. Both doses similarly reduced the rate of ischemic events versus placebo. The pharmacokinetics and pharmacodynamics of ticagrelor 60 mg bid have not been studied.

OBJECTIVES In this study, the authors sought to study the pharmacokinetics and pharmacodynamics for ticagrelor 60 mg compared with 90 mg bid.

METHODS A total of 180 patients who received >4 weeks of study medication had blood sampling in the morning pre-maintenance dose and again 2 h post-dose. All patients received aspirin. Plasma levels of ticagrelor and its active metabolite AR-C124910XX were determined. $P2Y_{12}$ inhibition was assessed by the VerifyNow P2Y12 assay (Accumetrics, Inc., San Diego, California) ($P2Y_{12}$ reaction units [PRU]), light transmittance aggregometry (adenosine diphosphate 5 and 20 μmol/l and arachidonic acid), and vasodilator-stimulated phosphoprotein phosphorylation assays. VerifyNow Aspirin assays and serum thromboxane B_2 measurements were performed.

RESULTS Mean pre- and post-dose plasma levels of ticagrelor were 35% and 38% lower, respectively, with 60 mg versus 90 mg. Both doses achieved high levels of platelet inhibition pre- and post-dose, with numerically slightly more variability with 60 mg: mean (SD) pre-dose PRU values were 59 ± 63 and 47 ± 43 for ticagrelor 60 and 90 mg, respectively ($p = 0.34$). High platelet reactivity, determined as PRU >208, was rare with the 60-mg pre-dose and was absent post-dose. Platelet reactivity pre- and post-dose, as measured by light transmittance aggregometry or vasodilator-stimulated phosphoprotein assays, was numerically but not significantly lower with 90 mg than with 60 mg. Aspirin response was not affected by either dose.

CONCLUSIONS Ticagrelor 60 mg bid achieved high levels of peak and trough platelet inhibition in nearly all patients, similar to that with 90 mg bid, helping to explain the efficacy of the lower ticagrelor dose in PEGASUS-TIMI 54. (J Am Coll Cardiol 2016;67:1145–54) © 2016 by the American College of Cardiology Foundation. Published by Elsevier. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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ABBREVIATIONS AND ACRONYMS

ACS = acute coronary syndrome

ADP = adenosine diphosphate

bid = twice a day

LTA = light transmittance aggregometry

MI = myocardial infarction

PD = pharmacodynamic

PK = pharmacokinetic

PRI = platelet reactivity index

PRU = $P2Y_{12}$ reaction units

Ticagrelor is a reversibly-binding $P2Y_{12}$ receptor antagonist that acts directly on platelet $P2Y_{12}$ receptors without requiring metabolic activation, although its active metabolite AR-C124910XX has similar potency at $P2Y_{12}$ (1). The extent of platelet $P2Y_{12}$ inhibition, therefore, reflects the plasma levels of ticagrelor and AR-C124910XX, because platelet inhibition recovers as these levels fall (2). Ticagrelor at a maintenance dose of 90 mg twice a day (bid) was shown to provide greater and more consistent platelet $P2Y_{12}$ inhibition than clopidogrel in patients with stable coronary artery disease (2) and acute coronary syndromes (ACS) (3,4). The PLATO (Platelet Inhibition and Patient Outcomes) trial demonstrated the superior efficacy of ticagrelor, given at a maintenance dose of 90 mg bid, compared with clopidogrel for up to 1 year in patients with ACS (5). Consequently, this regimen of ticagrelor is recommended in international guidelines as first-line therapy for up to 1 year following either non-ST-segment elevation ACS (6,7) or ST-segment elevation myocardial infarction managed with primary percutaneous coronary intervention (8,9).

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The PEGASUS-TIMI 54 (Prevention of Cardiovascular Events in Patients with Prior Heart Attack Using Ticagrelor Compared to Placebo on a Background of Aspirin-Thrombolysis In Myocardial Infarction 54) trial determined the benefit of long-term treatment with ticagrelor compared with placebo in aspirin-treated patients who had a history of myocardial infarction (MI) between 1 and 3 years prior to randomization and who also had additional risk factors for recurrent atherothrombotic events (NCT01225562) (10,11). Ticagrelor was studied at 2 doses: either 90 mg bid, as studied previously and approved for use following ACS, or 60 mg bid, which had not been studied previously. On the basis of modeling, it was assumed that the 60 mg bid dose would achieve less platelet inhibition than the 90 mg bid dose, but greater platelet inhibition and with less variability than clopidogrel 75 mg daily (10). However, no actual pharmacokinetic (PK) or pharmacodynamic (PD) data were available.

AstraZeneca and Medtronic. Dr. Angiolillo has received consulting fees or honoraria from Sanofi, Eli Lilly, Daiichi-Sankyo, The Medicines Company, AstraZeneca, Merck, Abbott Vascular, and PLx Pharma; has received payment for participation in review activities from CeloNova, Johnson & Johnson, and St. Jude Medical; and has received institutional grants from GlaxoSmith-Kline, Eli Lilly, Daiichi-Sankyo, The Medicines Company, AstraZeneca, Janssen Pharmaceuticals, Inc., Osprey Medical, Inc., Novartis, CSL Behring, and Gilead. Dr. Bonaca has received research grants through Brigham and Women's Hospital from AstraZeneca, Merck, Bayer, Roche Diagnostics, and GlaxoSmithKline; and has served as a consultant for AstraZeneca and Merck. Dr. Ahsan has received consultancy fees from Philips; and has received travel support from Daiichi-Sankyo and Boehringer Ingelheim. Dr. Bhatt has served on the advisory board of Cardax, Elsevier Practice Update Cardiology, Medscape Cardiology, and Regado Biosciences; is on the Board of Directors of Boston VA Research Institute and the Society of Cardiovascular Patient Care; is chair of the American Heart Association Quality Oversight Committee; has served on Data Monitoring Committees for Duke Clinical Research Institute, Harvard Clinical Research Institute, Mayo Clinic, and the Population Health Research Institute; has received honoraria from the American College of Cardiology (Senior Associate Editor, *Clinical Trials and News*, ACC.org), Belvoir Publications (Editor-in-Chief, *Harvard Heart Letter*), Duke Clinical Research Institute (clinical trial steering committees), Harvard Clinical Research Institute (clinical trial steering committee), HMP Communications (Editor-in-Chief, *Journal of Invasive Cardiology*), *Journal of the American College of Cardiology* (guest editor; associate editor), Population Health Research Institute (clinical trial steering committee), Slack Publications (Chief Medical Editor, *Cardiology Today's Intervention)*, Society of Cardiovascular Patient Care (secretary/treasurer), and WebMD (CME steering committees); is Deputy Editor of Clinical Cardiology; is vice-chair of the ACTION Registry Steering Committee; has received research funding from Amarin, AstraZeneca, Bristol-Myers Squibb, Eisai, Ethicon, Forest Laboratories, Ischemix, Medtronic, Pfizer, Roche, Sanofi, and The Medicines Company; has received royalties from Elsevier (Editor, *Cardiovascular Intervention: A Companion to Braunwald's Heart Disease*); is site coinvestigator for Biotronik and St. Jude Medical; is a trustee of the American College of Cardiology; and has performed unfunded research for FlowCo, PLx Pharma, and Takeda. Dr. Steg has received a research grant (to INSERM U1148) from Sanofi and Servier; has received speaking or consulting fees from Amarin, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, CSL-Behring, Daiichi-Sankyo, GlaxoSmithKline, Janssen, Lilly, Merck, Novartis, Pfizer, Regeneron, Roche, Sanofi, Servier, and The Medicines Company; and owns stocks in Aterovax. Dr. Cohen has received research grants from AstraZeneca; and is on the Speakers Bureau for AstraZeneca. Dr. Braunwald has received research grant support through Brigham and Women's Hospital from AstraZeneca, Merck, Daiichi-Sankyo, GlaxoSmithKline, Duke University, Bristol-Myers Squibb, Johnson & Johnson, Novartis, and Sanofi; has served as a consultant for Merck, Novartis, Sanofi, Theravance, and The Medicines Company; and has received lecture fees from Daiichi-Sankyo, Menarini International, Bayer, and Medscape. Dr. Sabatine has received research grant support through Brigham and Women's Hospital from Abbott Laboratories, Amgen, AstraZeneca, Critical Diagnostics, Daiichi-Sankyo, Eisai, Gilead, GlaxoSmithKline, Intarcia, Merck, Roche Diagnostics, Sanofi, and Takeda; and has served as a consultant for Alnylam, Amgen, AstraZeneca, CVS Caremark, Intarcia, and Merck. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Manuscript received October 13, 2015; revised manuscript received December 2, 2015, accepted December 14, 2015.

In PEGASUS-TIMI 54, both doses of ticagrelor had similar efficacy and safety compared with placebo: hazard ratios for the primary efficacy endpoint (the composite of cardiovascular death, MI, or stroke) were 0.85 (95% confidence interval [CI]: 0.75 to 0.96) and 0.84 (95% CI: 0.74 to 0.95) and for the primary safety endpoint (TIMI [Thrombolysis In Myocardial Infarction] major bleeding) were 2.69 (95% CI: 1.96 to 3.70) and 2.32 (95% CI: 1.68 to 3.21) for ticagrelor 90 and 60 mg bid, respectively (11). We performed a substudy in patients enrolled in PEGASUS-TIMI 54 to study the PK and PD effects of ticagrelor 60 mg bid and compare these with the effects of ticagrelor 90 mg bid.

METHODS

STUDY DESIGN. A total of 180 patients who were taking part in the PEGASUS-TIMI 54 study provided additional consent to take part in this substudy, which was conducted at 4 centers: 3 in the United Kingdom (Sheffield, Rotherham, and Nottingham) and 1 in the United States (Jacksonville, Florida). The substudy was conducted according to a protocol approved by the relevant ethics committee and institutional review board. Patients were studied after they had received study medication for at least 4 weeks following randomization in a 1:1:1 fashion to receive placebo, ticagrelor 60 mg bid, or ticagrelor 90 mg bid. Patients attended in the morning for their substudy visit, having taken their last dose of scheduled study medication the evening before. Venous blood was collected by venipuncture, then the morning dose of study medication was administered, and further venous blood was collected 2 h later. PK and PD analyses were performed as described in the following sections.

PK STUDIES. Venous blood was collected into lithium heparin tubes and placed on ice prior to centrifugation at 1,500 g within 30 min. Plasma was transferred to a polypropylene tube and immediately frozen at −20°C before being shipped to Covance Central Laboratories (Indianapolis, Indiana) for analysis of ticagrelor and AR-C124910XX levels (12). The lower limits of detection of the assays are 1 ng/ml for ticagrelor and 2.5 ng/ml for AR-C124910XX.

VERIFYNOW P2Y12 AND ASPIRIN ASSAYS. Venous blood was collected into 2-ml citrate Vacutainers and analyzed using the VerifyNow P2Y12 and Aspirin assays (Accumetrics, Inc., San Diego, California) according to the manufacturer's instructions (4). For the P2Y12 assay, $P2Y_{12}$ reaction units (PRU) and percentage inhibition (derived from the simulated baseline response induced by thrombin receptor-activating peptide) were recorded. The aspirin assay was performed in a subset of patients and aspirin response units were recorded.

LIGHT TRANSMITTANCE AGGREGOMETRY. Light transmittance aggregometry (LTA) was performed using a Chrono-log (Havertown, Pennsylvania) aggregometer, and adenosine diphosphate (ADP) (5 and 20 μmol/l) and arachidonic acid (1 mmol/l; performed post-dose in a subset of patients) as agonists (3). Maximum percentage LTA responses were recorded.

VASODILATOR-STIMULATED PHOSPHOPROTEIN PHOSPHORYLATION ASSAY. Aliquots of whole blood were processed using a vasodilator-stimulated phosphoprotein phosphorylation assay kit, and platelet reactivity index (PRI) was determined according to the manufacturer's instructions (BioCytex, Marseille, France) (4).

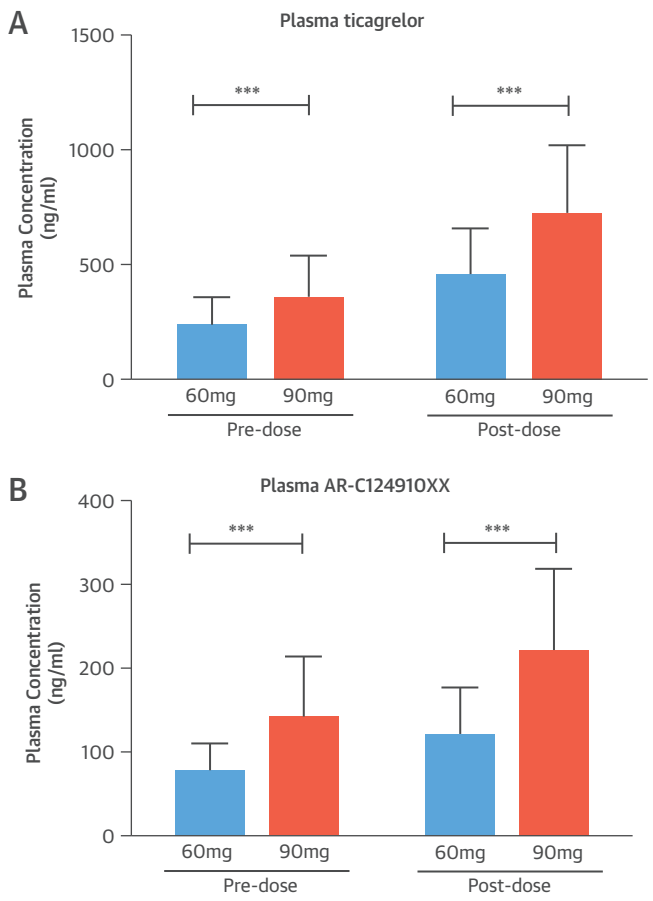
TABLE 1 Demographic Characteristics at Randomization and Medication at the Time of Blood Sampling After More Than 28 Days of Study Drug

	Placebo (n = 64)	Ticagrelor 60 mg (n = 58)	Ticagrelor 90 mg (n = 58)
Age, yrs	64.2 ± 6.6	63.3 ± 6.6	64.2 ± 6.9
Female	14 (22)	5 (9)	10 (17)
Body weight, kg	86 (75–98)	86 (76–100)	81 (71–94)
Body mass index, kg/m^2	29.7 ± 4.8	30 ± 5.6	28.6 ± 5.0
Race			
White	60 (94)	53 (91)	55 (95)
Black	4 (6)	5 (9)	3 (5)
Asian or other	0 (0)	0 (0)	0 (0)
Prior MI diagnosis			
Years since MI	1.7 (1.2–2.1)	1.7 (1.4–2.2)	1.8 (1.4–2.2)
STEMI	37 (58)	30 (52)	29 (50)
NSTEMI	27 (42)	28 (48)	28 (48)
Not known	0 (0)	0 (0)	1 (2)
Cardiovascular risk factors			
Current smoker	8 (13)	9 (16)	11 (19)
Diabetes mellitus	18 (28)	20 (35)	12 (21)
Hypertension	37 (58)	26 (45)	30 (52)
Multivessel CAD	41 (64)	37 (64)	32 (55)
Peripheral arterial disease	2 (3)	4 (7)	0 (0)
Creatinine clearance <60 ml/min	6 (9)	5 (9)	9 (16)
Time since last dose of study medication, pre-dose, h	12 (11–13)	12 (11–13)	12 (11–13)
Concomitant medication			
Aspirin 75 or 81 mg daily	64 (100)	58 (100)	58 (100)
Beta-blockers	59 (92)	48 (83)	53 (91)
Statins	61 (95)	56 (97)	55 (95)
ACE inhibitors or ARBs	60 (94)	52 (90)	52 (90)

Values are mean ± SD, n (%), or median (interquartile range). All comparisons between the groups are not significant.

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; CAD = coronary artery disease; MI = myocardial infarction; NSTEMI = non-ST-segment elevation myocardial infarction; STEMI = ST-segment elevation myocardial infarction.

FIGURE 1 Plasma Levels of Ticagrelor and its Active Metabolite



Mean levels of **(A)** ticagrelor and **(B)** AR-C124910XX in the ticagrelor 60-mg bid group (n = 57 to 58) and ticagrelor 90-mg bid group (n = 56 to 57). **Error bars** indicate SD. ***p ≤ 0.0001. bid = twice a day.

SERUM THROMBOXANE B_2. Venous blood was added to a serum separator tube (Becton Dickinson, Oxford, United Kingdom) and incubated at 37°C for 30 min followed by centrifugation at 1,000 g for 15 min. The supernatant serum was then transferred to cryovials and was stored at −80°C prior to analysis. Thromboxane B_2 levels were measured by an ELISA kit (Cayman Chemicals, Ann Arbor, Michigan).

SAMPLE SIZE AND STATISTICAL ANALYSIS. On the basis of expected mean pre-dose PRU values in the ticagrelor 90-mg group of 79 PRU and SD of 78 PRU (4), 57 patients were required in each ticagrelor group to provide 80% power to detect a mean pre-dose PRU value in the ticagrelor 60-mg group that was 50 PRU higher than for the 90-mg group with an alpha of 0.01. A total of 180 patients were included to allow for expected imbalance in the final numbers in each of the 3 groups.

Data were analyzed using SAS version 9.3 (SAS Institute, Cary, North Carolina) and expressed as mean and SD or median and interquartile range, as indicated. Continuous data for the placebo and ticagrelor groups were compared using the Kruskal-Wallis test. Categorical variables were compared using the Fisher exact test. Statistical significance was attached to p values <0.01 to allow for multiple group comparisons.

The proportion of patients in each treatment group with responses greater than thresholds previously associated with increased ischemic risk ("high platelet reactivity") (13) were determined as follows: VerifyNow P2Y12 assay result >208 PRU (14,15); maximum LTA response to ADP 20 μmol/l >59% (16–18); maximum LTA response to ADP 5 μmol/l >46% (17,18); PRI >50% (18,19); VerifyNow aspirin response units >550 (20,21); and maximum LTA response to arachidonic acid ≥20% (22,23).

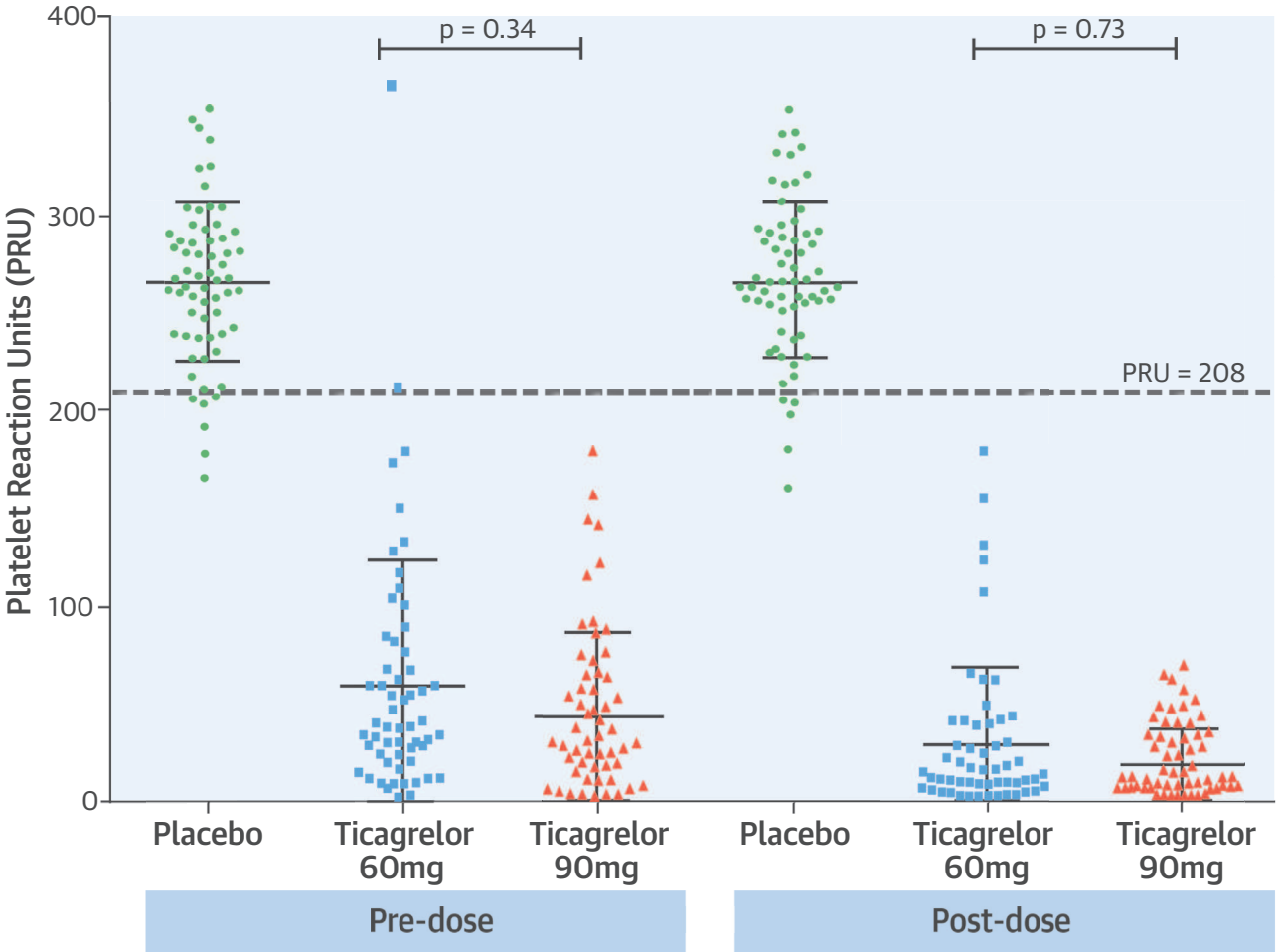
RESULTS

STUDY POPULATION. A total of 180 patients were studied after at least 4 weeks of maintenance treatment with placebo (n = 64), ticagrelor 60 mg bid (n = 58), or ticagrelor 90 mg bid (n = 58). Demographic characteristics and comedications were generally well matched in the 3 treatment arms (**Table 1**). All patients were receiving low-dose aspirin. The baseline characteristics of the substudy patients versus the entire trial cohort are shown in Online Table 1.

PK RESULTS. Mean pre- and post-dose plasma ticagrelor levels in the 60-mg group were 65% and 62% of those in the 90-mg group, respectively, and mean pre- and post-dose plasma AR-C124910XX levels in the 60-mg group were both 54% of those in the 90-mg group (**Figure 1**).

MEASURES OF PLATELET $P2Y_{12}$ INHIBITION. All measures of $P2Y_{12}$-mediated platelet reactivity showed numerically (but not statistically significantly) greater platelet inhibition with ticagrelor 90 mg bid compared with ticagrelor 60 mg bid, both pre- and post-dose (**Central Illustration**, **Figures 2 to 4**, Online Table 2). VerifyNow P2Y12 assay results showed high mean levels of platelet inhibition in both ticagrelor groups, but a slightly greater SD with the 60 mg bid regimen (**Central Illustration**, **Figure 2**, Online Table 2): mean pre-dose PRU values were 59 ± 63 PRU versus 47 ± 43 PRU for the 60 mg versus 90 mg ticagrelor doses, and post-dose values were 29 ± 39 PRU versus 20 ± 19 PRU, respectively.

CENTRAL ILLUSTRATION **Platelet Inhibition With Ticagrelor 60 mg Compared With 90 mg Twice Daily: VerifyNow P2Y12 Assay Results**



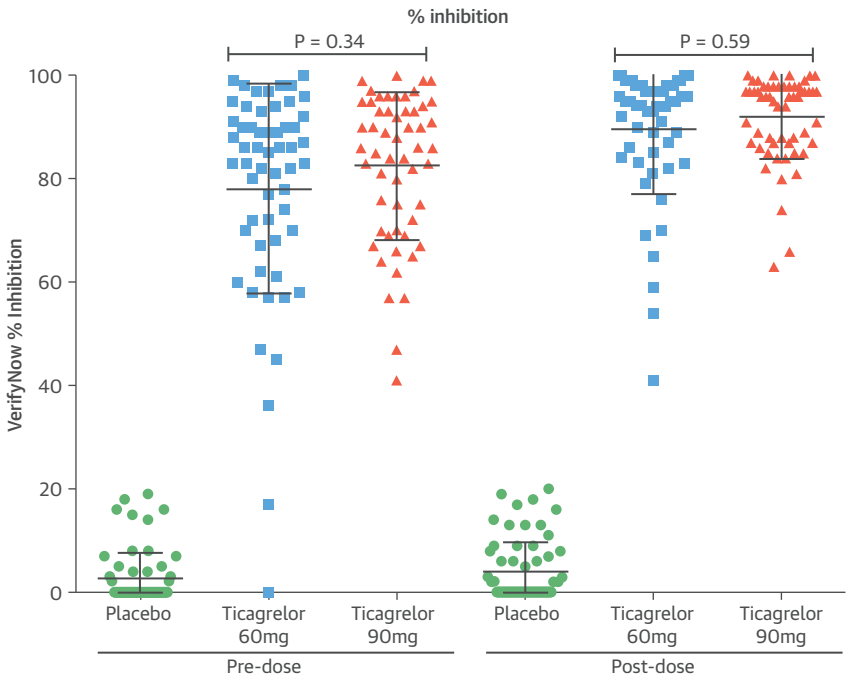
Storey, R.F. et al. J Am Coll Cardiol. 2016; 67(10):1145-54.

Individual results for the VerifyNow P2Y12 assay (Accumetrics, Inc., San Diego, California) expressed as $P2Y_{12}$ reaction units (PRU). The **dashed line** indicates a level of 208 PRU as a threshold for high platelet reactivity. **Solid lines with error bars** indicate mean ± SD. There are no significant differences between the ticagrelor groups (all $p > 0.01$).

Similarly, mean LTA responses to ADP (Figure 3) and mean PRI levels (Figure 4) were low in both ticagrelor groups compared with the placebo group at both pre- and post-dose. The VerifyNow P2Y12 percent inhibition estimation provided the greatest separation between placebo group and ticagrelor group values (Figure 2).

HIGH $P2Y_{12}$-MEDIATED PLATELET REACTIVITY. Overall, high platelet reactivity with the measures of $P2Y_{12}$ inhibition was uncommon (Central Illustration, Figures 3 and 4, Table 2). With the VerifyNow P2Y12 assay, only 2 patients in the ticagrelor 60-mg group had PRU values >208 pre-dose (3.5%), and there were no other instances of high platelet reactivity (Central Illustration). One of these patients also had high platelet reactivity to ADP as determined by LTA, and both had high platelet reactivity as determined by vasodilator-stimulated phosphoprotein phosphorylation assay. The patient with a pre-dose PRU value of 369 (0% inhibition) had PK levels of both ticagrelor and AR-C124910XX that were below the limits of detection, indicating poor compliance, and this patient's post-dose value was 122 PRU (69% inhibition). Another patient with a pre-dose PRU value of 211 (17% inhibition) also had low PK levels of ticagrelor

FIGURE 2 VerifyNow P2Y12 Assay % Inhibition Results



Individual results for the VerifyNow P2Y12 assay (Accumetrics, Inc., San Diego, California) expressed as percentage inhibition derived from an estimated "baseline" response using thrombin receptor-activating peptide as agonist. **Solid lines with error bars** indicate mean ± SD. There are no significant differences between the ticagrelor groups (all $p > 0.01$).

(40 ng/ml) and AR-C124910XX (18 ng/ml) and had a post-dose value of 39 PRU (86% inhibition).

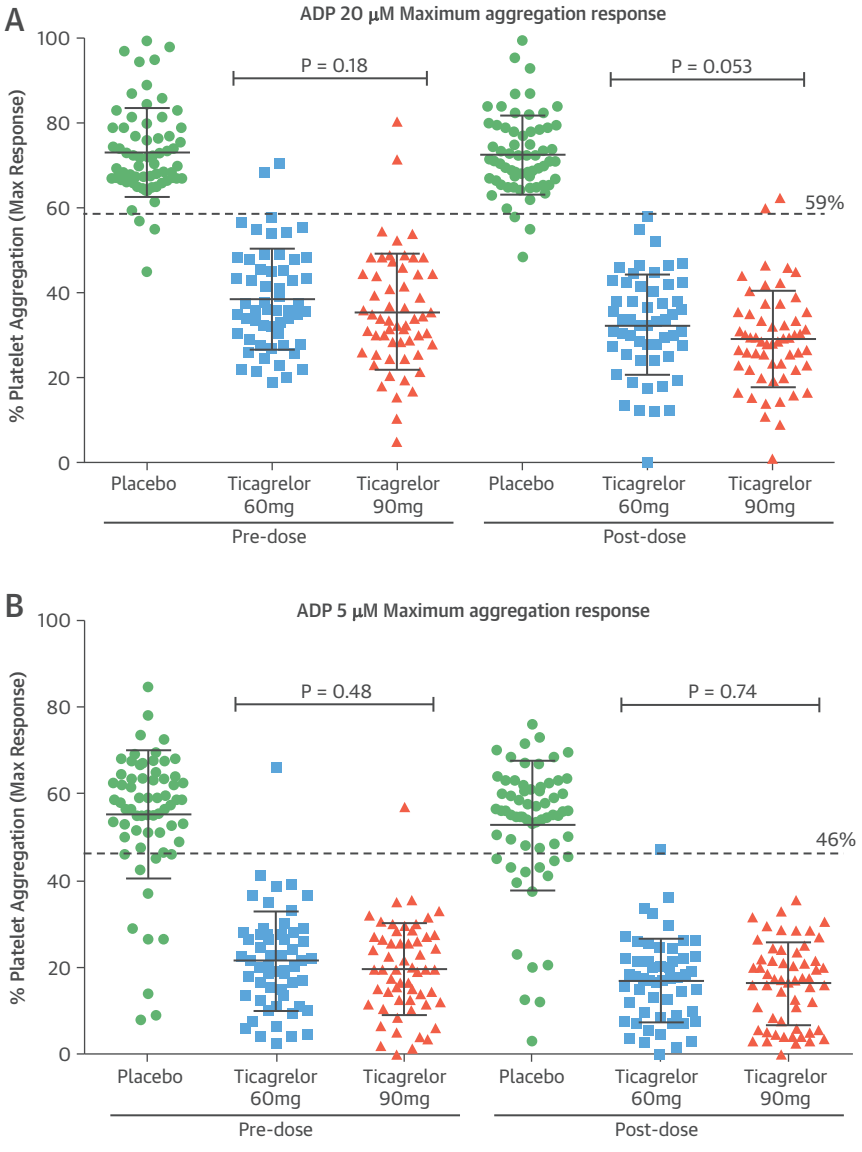
There were few cases of high platelet reactivity in either ticagrelor group according to either LTA responses to ADP or PRI levels (Figures 3 and 4, Table 2).

MEASURES OF ASPIRIN RESPONSE. VerifyNow Aspirin response assay, LTA with arachidonic acid as agonist, and serum thromboxane B_2 levels all indicated high levels of cyclooxygenase-1 inhibition by aspirin in nearly all patients, with few patients having high platelet reactivity to arachidonic acid in each of the treatment groups pre- and post-ticagrelor dose (Online Figure 1, Online Table 3). There were no significant differences between the placebo group and either of the ticagrelor groups.

DISCUSSION

This PEGASUS-TIMI 54 substudy is the first assessment of the PK and PD effects of the ticagrelor 60 mg bid regimen. The PK results showed levels of ticagrelor in the 60-mg group at approximately two-thirds of the ticagrelor levels in the 90-mg group, as expected on the basis of previous PK studies. Most intriguing was the similar mean platelet $P2Y_{12}$ inhibition achieved with ticagrelor 60 and 90 mg bid. This was unexpected, as it had been intended to achieve a lower level of platelet inhibition with the 60-mg regimen (10), but the observed similarity in platelet inhibition between the 2 ticagrelor groups is entirely consistent with the efficacy results in the main trial being almost identical with ticagrelor 60 and 90 mg bid compared with placebo (11). Indeed, only 2 patients in the ticagrelor 60-mg group had high platelet reactivity pre-dose, according to the VerifyNow P2Y12 assay, and both of these patients had excellent inhibition post-dose. Furthermore, 1 of these patients had evidence of poor compliance with study medication. The tolerability of the 60-mg bid regimen is slightly better than with the 90-mg bid regimen: the frequency of dyspnea adverse events related to ticagrelor is moderately associated with dose and PK levels (24–27), and the rates of dyspnea were numerically lower with ticagrelor 60 mg bid compared with ticagrelor 90 mg bid in PEGASUS-TIMI 54 (11). In addition, the rates of bleeding, transfusion,

FIGURE 3 **ADP-Induced Platelet Aggregation**

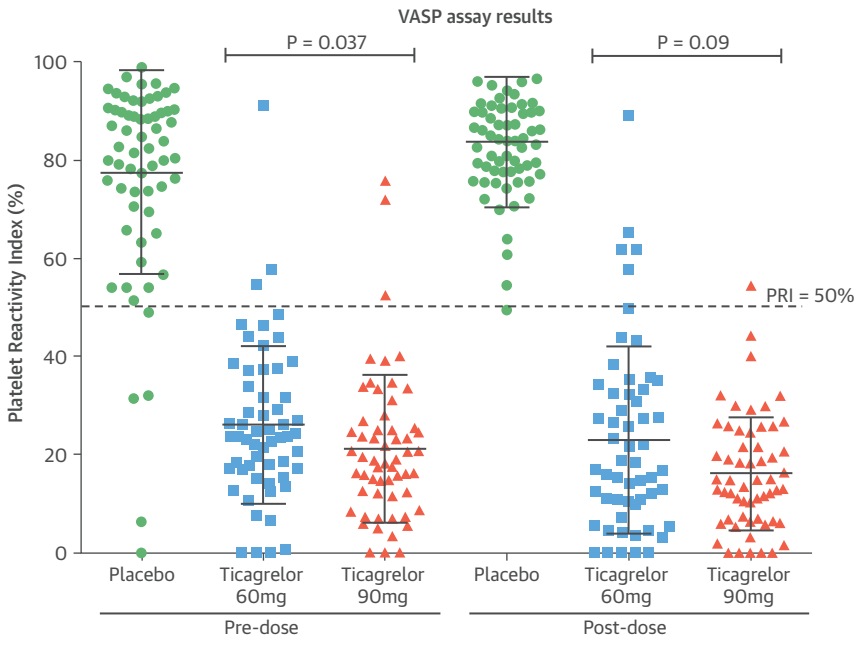


Individual results for the platelet aggregation measured by light transmittance aggregometry and assessing maximum aggregation response to **(A)** ADP 20 μmol/l and **(B)** ADP 5 μmol/l. The **dashed lines** indicate threshold values for high platelet reactivity. **Solid lines with error bars** indicate mean ± SD. There are no significant differences between the ticagrelor groups (all $p > 0.01$). ADP = adenosine diphosphate.

and gout were numerically lower with ticagrelor 60 mg bid compared with ticagrelor 90 mg bid in PEGASUS-TIMI 54. Our results therefore support the preferential use of ticagrelor 60 mg bid instead of 90 mg bid in patients with good adherence to antiplatelet medication who are more than 1 year post-MI. This approach has been endorsed by the recent Food and Drug Administration approval of ticagrelor 60 mg bid for long-term use following MI (28). Furthermore, a therapeutic trial of the 60-mg ticagrelor dose might be considered prior to 1 year post-MI for those who cannot tolerate the 90-mg dose due to dyspnea or minor bleeding.

We observed no effect of ticagrelor on the measurements of aspirin response, suggesting that, at least in aspirin-treated patients, $P2Y_{12}$ inhibition does not significantly influence release of the platelet agonist thromboxane A_2 (because PD measures of

FIGURE 4 VASP Phosphorylation Assay Results



Individual results for the VASP phosphorylation assay. The **dashed line** indicates the threshold value of 50% for high platelet reactivity. **Solid lines with error bars** indicate mean ± SD. There are no significant differences between the ticagrelor groups (all $p > 0.01$). PRI = platelet reactivity index; VASP = vasodilator-stimulated phosphoprotein.

aspirin response reflect conversion of arachidonic acid into thromboxane A_2). This raises a note of caution about dropping aspirin when treating with ticagrelor alone if there is a high thrombotic risk and emphasizes the importance of waiting for the results of adequately powered clinical outcomes studies of ticagrelor monotherapy compared with dual antiplatelet therapy.

TABLE 2 Proportions of Patients With High $P2Y_{12}$-Mediated Platelet Reactivity According to Pre-Defined Threshold Values

	Ticagrelor 60 mg		Ticagrelor 90 mg		p Value
Measures of $P2Y_{12}$ Inhibition	Sample Size	Proportion	Sample Size	Proportion	90 mg vs. 60 mg
VerifyNow P2Y12 assay					
Pre-dose, PRU >208	57	2 (4)	55	0 (0)	0.50
Post-dose, PRU >208	55	0 (0)	58	0 (0)	N/A
VASP assay					
Pre-dose, PRI >50%	57	3 (5)	58	3 (5)	1.0
Post-dose, PRI >50%	56	5 (9)	58	1 (2)	0.11
LTA, 20 μmol/l ADP					
Pre-dose, aggregation >59%	56	2 (4)	57	2 (4)	1.0
Post-dose, aggregation >59%	54	0 (0)	57	2 (4)	0.50
LTA, 5 μmol/l ADP					
Pre-dose, aggregation >46%	56	1 (2)	57	1 (2)	1.0
Post-dose, aggregation >46%	54	1 (2)	57	0 (0)	0.49

Sample size is the number of patients with available data in each group. Proportion is the number with values above the given threshold value, given as n (%) (percent is calculated as: [sample size/proportion] × 100).

ADP = adenosine diphosphate; LTA = light transmittance aggregometry; N/A = not applicable; PRU = $P2Y_{12}$ reaction units; VASP = vasodilator-stimulated phosphoprotein.

STUDY LIMITATIONS. This PK/PD substudy was not large enough to allow assessment of the relationship between platelet function results and clinical outcomes. We did not assess the effects of the 2 ticagrelor regimens on adenosine reuptake, and so cannot exclude a significant difference between the 2 ticagrelor regimens on plasma adenosine levels, which may also contribute to platelet inhibition in vivo (29,30); this is being assessed in a further study of patients undergoing elective percutaneous coronary intervention (NCT02327624). We also did not assess baseline platelet reactivity prior to initiation of study medication; we relied on the VerifyNow P2Y12 assay estimate of inhibition, using thrombin receptor activating peptide-induced response to give a predicted baseline response, rather than deriving inhibition data from pre-treatment platelet responses. The substudy did not include Asian patients and had limited numbers of low body weight patients (<60 kg), so further PK/PD studies are required to assess these groups.

CONCLUSIONS

We have shown that ticagrelor 60 mg bid achieves high levels of peak and trough platelet inhibition in patients with a prior history of MI, with similar mean levels of inhibition compared with 90 mg bid. These results help to explain the comparable efficacy of the lower ticagrelor dose in the PEGASUS-TIMI 54 study.

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PERSPECTIVES

COMPETENCY IN PATIENT CARE AND PROCEDURAL SKILLS:

In patients with prior MI receiving extended dual antiplatelet therapy, ticagrelor 60 mg bid provides a degree of platelet inhibition comparable to that achieved with 90 mg bid. This may explain the nearly equivalent efficacy of these doses for prevention of recurrent ischemic events.

TRANSLATIONAL OUTLOOK: These observations call for further investigation of the safety and efficacy of 60-mg bid dosing of ticagrelor in other clinical settings associated with atherothrombosis.

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KEY WORDS aspirin, coronary artery disease, $P2Y_{12}$ receptor

APPENDIX For supplemental Methods as well as supplemental tables and figures, please see the online version of this article.