High on-treatment platelet reactivity and P2Y12 antagonists in clinical trials

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Summary
Dual antiplatelet therapy with aspirin and clopidogrel in patients undergoing percutaneous coronary intervention (PCI) and in patients with acute coronary syndromes (ACS) has substantially decreased the rate of cardiovascular events. Within the past decade, the variability in pharmacodynamic response as well as the moderate antiplatelet efficacy of clopidogrel has raised major concerns, since high on-clopidogrel platelet reactivity has consistently been associated with increased risk for ischaemic events in PCI patients. The variability in response could be linked to genetic polymorphisms impacting on activity of cytochrome P450 enzymes as well as clinical and demographic variables, but, taken together, factors identified so far can explain only up to approximately 12% of this variability in adenosine diphosphate-induced platelet aggregation on clopidogrel. Regulatory agencies as well as major cardiac societies suggest the use of other anti-platelet medications or alternative dosing strategies for clopidogrel in patients with reduced effectiveness of clopidogrel. This review will focus on the current status of alternate strategies for more sufficient suppression of high platelet reactivity.

Keywords
P2Y12 antagonists, platelet function, clopidogrel

Introduction
Platelet activation and aggregation play pivotal pathophysiological roles in the development of ischaemic events during and after acute coronary syndromes (ACS) and percutaneous coronary interventions (PCI) (1). Acetylsalicylic acid (aspirin) was the first antiplatelet drug with proven benefit in ACS (2-4). Studies demonstrating significant platelet activation in ACS and during PCI despite treatment with aspirin and anticoagulant regimens provided the rationale for clinical studies investigating novel antiplatelet strategies (5, 6). The superiority of dual antiplatelet therapy with aspirin and a P2Y12 receptor antagonist compared with intense oral anticoagulation was shown in the mid-1990s. Compared with aspirin plus vitamin K antagonists, dual antiplatelet therapy with aspirin and the thienopyridine ticlopidine reduced the incidence of both cardiac and haemorrhagic events as well as vascular complications after the placement of coronary artery stents (7). The clinical use of ticlopidine is hampered by major haematological side effects. This led to the development of clopidogrel, another member of the thienopyridine class of antiplatelet drugs. In patients after PCI, clopidogrel has similar antiplatelet efficacy despite the use of lower doses during chronic treatment with the major advantage of a more benign side-effect profile compared with ticlopidine (8). The improved tolerability of clopidogrel enabled administration of loading doses in the range of 300 to 600 mg by which the maximum antiplatelet effect of the drug can be obtained within 2-6 hours (h) after dosing (9). Thus for more than 15 years, dual antiplatelet therapy with aspirin and clopidogrel was the standard of care for prevention of ischaemic complications after PCI and in patients with ACS (10, 11).

Although already approved as early as 1997 in the US, antiplatelet efficacy of clopidogrel has been extensively investigated within the last decade. Studies were stimulated by the development of new user-friendly laboratory methods for assessment of platelet function and the widespread clinical use of clopidogrel.

The initial observation that the pharmacodynamic response to clopidogrel is associated with a large inter-individual variability (Figure 1) raised three key questions:

- Which mechanisms(s) are responsible for the variability in effect?
- What is the clinical impact of the variability in response?
- Which therapeutic strategies can be used to achieve a more consistent and predictable antiplatelet effect?

Mechanisms(s) responsible for variability in antiplatelet effect of clopidogrel

Several mechanisms have been identified to contribute to the observed variability in antiplatelet response to clopidogrel. Current

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research has hereby focused on the contribution of baseline clinical and demographic factors, drug-drug interactions and polymorphisms in drug metabolising enzymes and drug transporters to the variability in response to clopidogrel.

**Baseline clinical and demographic factors**

Various clinical and demographic variables have been identified that were associated with the response to clopidogrel in clinical studies. Geisler et al. (12) investigated in a large cohort of 1,092 patients with symptomatic coronary artery disease (both stable angina patients and patients with ACS) treated with coronary stenting the impact of various clinical and demographic factors on residual platelet aggregation after administration of a 600 mg loading dose of clopidogrel. Age >65 years, diabetes mellitus, reduced left ventricular function, renal failure (serum creatinine >1.5 mg/dl) and presentation with ACS were identified as predictors of high on-clopidogrel platelet reactivity. They allocated weighing factors in the range of 1 to 3 to these variables to account for differences in impact. Thereby, diabetes was weighted by factor 2, ACS by factor 1, severe left ventricular dysfunction by factor 3, age >65 years by factor 1, and renal failure by factor 2 yielding in a maximum PREDICT score of 9. Patients with a score level 7-9 had an odds ratio (OR) of 2.7 for a platelet aggregation in the upper tertile of the cohort. Patients in the upper tertile of aggregation incurred the highest incidence of cardiovascular events (cardiovascular death, myocardial infarction, ischaemic stroke) and death from any cause within short-term follow-up (30 days).

**Drug-drug interactions with clopidogrel**

The variable antiplatelet effect of clopidogrel can be attributed mainly to differences in the systemic availability of the active metabolite of clopidogrel (13, 14). Drug-drug interactions (15) and genetic polymorphisms determining the activity of the enzymes involved in metabolism of clopidogrel (16-18) are key factors impacting on systemic availability of the active metabolite of clopidogrel (19).

Clopidogrel is an inactive pro-drug requiring bioactivation into the active metabolite after absorption from the gastrointestinal tract (Figure 2). The metabolite formed is the only entity responsible for the inhibitory effect of the P2Y12 receptors. Unfortunately, the majority of clopidogrel absorbed is subject to the activity of carboxylesterase (CES) in plasma by which 85 to 90% of parent clopidogrel is hydrolysed into an inactive carboxyl metabolite (SR26,334). Only a minor proportion of the dose absorbed escapes inactivation by CES and is subject to oxidation by hepatic cytochrome (CYP) P450 enzymes resulting in a thiophene derivative (2-oxo-clopidogrel). Approximately 50% of the primary metabolite 2-oxo-clopidogrel is cleaved by esterases into an inactive carboxyl metabolite. Remaining 2-oxo-clopidogrel is metabolised in a second step by opening of the thiophene ring to the thiol metabolite (20-22). The cis-thiol metabolite inhibits irreversibly the platelet P2Y12 receptor via covalent binding of its sulphydryl group for the life-span of the platelet.

Various CYP P450 enzymes namely CYP2B6, CYP2C9, CYP3A4/5, CYP1A2 and CYP2C19 contribute to the formation of the active metabolite of clopidogrel. Polypharmacy is frequent in patients with coronary artery disease and the vast majority of cardiac drugs are eliminated via hepatic metabolism. Pharmacodynamic studies showing that lipophilic statins such as atorvastatin, proton pump inhibitors (PPI) such as omeprazole, calcium channel blockers like amiodipine, verapamil or diltiazem and several other drugs may attenuate antiplatelet response to clopidogrel and raise concerns regarding an increased risk for ischaemic events if patients were on concomitant treatment with these drugs (23). On the other hand, inducers of CYP3A4 like rifampicin may amplify antiplatelet efficacy of clopidogrel (24). There is, however, no consistent evidence from sufficiently powered prospective studies that clopidogrel-drug interactions have a detrimental impact on clinical outcome of patients after PCI. The initial finding of an attenuation of response to clopidogrel by lipophilic statins could not be confirmed in a large prospective study in 1,368 patients scheduled for coronary angiography (25). A randomised pharmacokinetic / pharmacodynamic study investigated in a cross-over design the interaction between omeprazole and clopidogrel (26). Concomitant administration of omeprazole decreased the systemic exposure to the active metabolite of clopidogrel and attenuated antiplatelet effects indicating a metabolic drug-drug interaction between clopidogrel and omeprazole. A subsequent prospective randomised trial compared a fixed combination of clopidogrel 75 mg + omeprazole 20 mg with clopidogrel 75 mg in patients with an indication for dual antiplatelet therapy (27). At 180 days, the rate of gastrointestinal bleeding was substantially decreased in patients randomised to clopidogrel plus omeprazole, and there was no apparent interaction between clopidogrel and omeprazole regarding cardiovascular endpoints. Unfortunately, the study was terminated prematurely after randomisation of 3,873 out of 5,000 planned patients due to lack of financial support. The clinical relevance of this...
pharmacologic interaction remains therefore uncertain but could be important in specific subgroups of patients especially in those at a higher thrombotic risk (28).

Calcium channel blockers (CCB) can also inhibit CYP3A4. Studies have investigated if the antiplatelet effect of clopidogrel is attenuated by CCB. A decrease in antiplatelet effect was observed in patients on concomitant treatment with amlopidine, while conflicting results were obtained for verapamil and diltiazem (29, 30, 18).

Cytochrome P450 and MDR-1 genetic polymorphisms
CYP2B6, CYP2C9, CYP3A4/5 and CYP1A2 contribute only to a minor extent to the formation of the active metabolite (20, 21). In contrast, CYP2C19 has a crucial role in the metabolism of clopidogrel (31, 32, 22). Several studies investigated the impact of genetic polymorphisms in CYP2C19 on antiplatelet effect of clopidogrel and the clinical relevance of this genetic background. Presence of the CYP2C19 681G>A (*2) polymorphism results in a complete loss of enzyme activity due to creation of an aberrant splice site (33). Moreover, the CYP2C19*2 allele is found in approximately 15% of Caucasians and Africans and an even higher proportion of the Asian population are carriers of this allele (34). Other CYP2C19 variants (*3 - *8) which are also associated with a reduced enzymatic activity have a lower allele frequency (<1%) and are therefore of minor clinical importance at least in the Caucasian population. Carriage of CYP2C19 loss-of-function (LOF) alleles has been shown to be associated with a decreased antiplatelet activity of clopidogrel (32, 17). Subsequent studies confirmed that patients undergoing PCI carrying CYP2C19 LOF alleles are at increased risk for adverse cardiovascular events (35, 19, 36, 37). Initially, there was a debate if the risk for ischaemic events is increased in CYP2C19 LOF homozygous patients only. A meta-analysis comprising 9,685 cardiac patients after PCI resolved this issue by showing a gene-dose effect with carriage of even one reduced-function CYP2C19 allele is already associated with a significant increased risk of major adverse cardiovascular events, particularly stent thrombosis (38).

Polymorphisms within the CYP2C19 system comprise not only loss-of-function alleles but also gain-of-function mutations (CYP2C19*17). This allele variant is responsible for an increased catalytic activity most likely due to a higher transcription rate of the gene (39). The clinical impact of this polymorphism regarding clopidogrel should result in a phenotype with increased antiplatelet effect which may potentially expose the patients to an increased risk of bleeding but results from studies are mixed. While three studies showed an independent association of CYP2C19*17 allele carriage with lower on-clopidogrel platelet reactivity (40-42), CYP2C19*17 was not associated with ADP-stimulated platelet aggregation in healthy Amish subjects treated with clopidogrel. However, two recently published meta-analyses confirmed that carriers of the CYP2C19*17 allele had a lower risk of cardiovascular events but an increased risk of bleeding (43, 44).

In conclusion, carriage of the CYP2C19*2 allele is the strongest individual factor contributing to variability in the ex vivo determined platelet-inhibitory effect of clopidogrel, but this polymorphism accounts only for 5.2 to 12 % of the variation of ADP-induced aggregation (45, 18). Since CYP2C19*2 carrier status together with all demographic and clinical predictors for high on-clopidogrel could only explain 11.5% of platelet reactivity on treatment with clopidogrel (18), search for further candidates to explain this puzzle is ongoing.

Clopidogrel is a substrate of the intestinal efflux transport pump P-glycoprotein which is encoded by the ABCB1 (MDR1) gene (46). In one study, patients with two variant alleles of ABCB1 (TT at nucleotide 3435) encoding for the intestinal efflux transport pump P-glycoprotein modulating clopidogrel absorption had a higher rate of cardiovascular events at one year compared to those with the ABCB1 wild-type genotype (37). A genome-wide association study (GWAS) identified only single-nucleotide polymorphisms on chromosome 10q24 within the CYP2C18–CYP2C19–CYP2C9–CYP2C8 cluster being associated with clopidogrel response and the ABCB1 variant was not associated with ADP-stimulated platelet aggregation after clopidogrel (45). Moreover, the genetic sub-study of the PLATO trial as well as

Figure 2: Metabolism of clopidogrel and cytochrome P450 enzymes involved (20-22).
a recent meta-analysis failed to confirm any association between ABCB1 C3435T polymorphism and the risk of ischaemic events (47, 48).

A recent study proposed that the enzyme paraoxonase-1 (PON-1) might play a key role instead of CYP2C19 in the second activation step of clopidogrel. It was suggested that 72.5% of the variability in antiplatelet might be attributed to the activity of this enzyme (49). However, several independent studies and a recent meta-analysis could not confirm the suggested association between activity of PON-1 and antiplatelet effect of clopidogrel or clinical outcome of patients after PCI (50-52).

New oral P2Y12 inhibitors

The regulatory agencies recently approved two new inhibitors of the P2Y12 receptor, prasugrel (2009) and ticagrelor (2011). The approved indications were derived from the study design of the respective large Phase III studies TRITON-TIMI 38 and PLATO (53, 54) in which the new compounds were compared with clopidogrel. Prasugrel is indicated for prevention of thrombotic cardiovascular events (incl. stent thrombosis) in patients with ACS managed with PCI. Ticagrelor is indicated to reduce the rate of thrombotic cardiovascular events in patients with ACS without the restriction of obligatory management by PCI since the PLATO trial also enrolled only medically managed ACS-patients.

Prasugrel is a thienopyridine derivative like ticlopidine and clopidogrel. Parent prasugrel is pharmacologically inactive and requires metabolism to form the active metabolite which similar to ticlopidine and clopidogrel causes an irreversible inhibition of the P2Y12 receptor. The chemical structure of prasugrel has been optimised to abolish the recognised weaknesses in metabolism of clopidogrel in which the vast majority of administered drug is shunted to inactive metabolites. Prasugrel undergoes rapid deacetylation by intestinal esterases with the formation of an inactive intermediate thiocarbonyl metabolite. The active metabolite is formed from the thiocarbonyl by oxidative metabolism with various isoenzymes of the cytochrome P450 system (CYP3A4/5, CYP2B6, CYP2C19, CYP2C9) contributing to this final step (55). Moreover, common loss-of-function CYP genetic variants do not impact on the systemic availability of the active metabolite, the antiplatelet efficacy nor on the ischaemic event rates in PCI-patients treated with prasugrel (56, 57). The much more efficient metabolic conversion explains the higher and substantially less variable inhibition of ADP-induced platelet aggregation achieved by prasugrel despite the use of much lower doses for loading (60 mg) and chronic therapy (10 (5) mg once daily [OD]) (58).

The cyclo-pentyl-triazolo-pyrimidine derivative ticagrelor is the first in the class of direct acting irreversible inhibitors of the P2Y12 receptor approved by the regulatory agencies (59). The major metabolite of ticagrelor (AR-C124910XX) is formed by metabolism via CYP3A4 and has also antiplatelet properties (60). The reversible inhibition of the P2Y12 receptor and the relatively short half-life of ticagrelor (8–12 h) require twice daily dosing (90 mg BID) to achieve a constant inhibition of ADP-induced platelet aggregation. As with prasugrel, the pharmacodynamic profile of ticagrelor is characterised by a more rapid onset of effect after loading doses of 180 mg with higher and less variable platelet inhibition achieved after loading doses as well as during maintenance therapy compared to clopidogrel (61). Regarding offset of effect, there was no significant difference in the extent of platelet inhibition between ticagrelor and clopidogrel at 24 and 48 h following the final dose, whereas ticagrelor had significantly less effect than clopidogrel at 72 and 120 h after the last dose (61).

Antiplatelet response and clinical outcome

The GOLD (AU-Assessing Ultegra) study determined platelet reactivity in 500 patients who received a glycoprotein (GP) IIb/IIIa inhibitor while undergoing PCI (62). Although, GP IIb/IIIa inhibitors are administered intravenously, a substantial variability in platelet inhibition was observed and suboptimal platelet inhibition was associated with a more than two-fold increase in ischaemic events.

Subsequently, studies with assessment of adenosine diphosphate (ADP) induced platelet aggregation reported substantial inter-individual variability in antiplatelet effect of clopidogrel both after loading doses in the range of 300 to 600 mg as well as on maintenance therapy using a 75 mg OD dosing regimen (63-65).

Barragan et al. (66) used the vasodilator-stimulated phosphorylation assay (VASP) phosphorylation assay (Biocytex, Marseille, France) for assessment of P2Y12 receptor inhibition on thienopyridines. Patients (n=16) who presented with a subacute stent thrombosis after PCI had a significantly higher on-treatment platelet reactivity (HPR) than control cases without stent thrombosis (n=30). The association between clopidogrel response and ischaemic events was investigated in patients undergoing primary PCI with stenting for acute ST-segment-elevation myocardial infarction (STEMI) using turbidimetric platelet aggregation (67). Patients were stratified into quartiles according to inhibition of ADP-induced aggregation. Patients in the quartile with the lowest inhibition of aggregation were called "clopidogrel resistant", and 40% of resistant patients suffered a cardiovascular event during six months follow-up after index PCI. Several studies confirmed subsequently the association between HPR and increased risk of ischaemic events, but these studies were limited to some extent by their low sample size. EXCELSIOR (Impact of Extent of Clopidogrel-Induced Platelet Inhibition During Elective Stent Implantation on Clinical Event Rate) was a large prospective study enrolling 802 patients after elective coronary stent placement (65). Patients were on chronic aspirin (≥100 mg OD) and a loading dose of clopidogrel 600 mg was administered before PCI. Patients in this low risk cohort with an ADP (5 µM)-induced residual platelet aggregation above the median (>14%) carried a more than six-fold risk for 30-day major adverse cardiac events.

A meta-analysis extracted the data from twenty observational studies which investigated the association between platelet reactivity and clinical outcome in 9,187 patients treated with aspirin and clopidogrel after PCI (68). Studies were eligible if ADP-specific
platelet function assays (ADP-stimulated light transmission aggregometry, VASP assay, VerifyNow P2Y12 test [Accumetrics, San Diego, CA, USA], or multiple electrode impedance aggregometry with ADP as stimulant [Roche Diagnostics, Mannheim, Germany]) were used. Despite significant differences in the methodology as well as in the definition of HPR between studies, HPR was a strong predictor of cardiovascular death (OR, 95% confidence interval [CI]: 3.35 [2.39-4.70]), myocardial infarction (MI; 3.00 [2.26-3.99]), stent thrombosis (ST; 4.14 [2.74-6.25]), and the composite endpoint of reported ischaemic events (4.95 [3.34-7.34]). A further meta-analysis tried to circumvent methodological issues caused by different platelet function assays by analysing only studies in which platelet reactivity on clopidogrel was assessed in patients after PCI by the VerifyNow P2Y12 point-of-care assay only (69). Data were extracted from six prospective studies enrolling 3,059 patients. A P2Y12 reaction unit (PRU) cut-off value of 230 was obtained by receiver-operating characteristic curve analysis with regard to death, MI or ST. A PRU-value ≥230 was associated with a more than two-fold increased risk for the combined cardiovascular endpoint of death, MI or ST.

A recently published consensus paper reviewed 28 independent studies which demonstrate a clear association between HPR after stimulation with ADP and adverse clinical events (70). Despite the large body of evidence for HPR being associated with an increased risk for ischaemic events throughout the whole spectrum of patients undergoing PCI in elective intervention to patients treated with primary PCI in STEMI, routine measurement of platelet reactivity has so far not been recommended in the guidelines of cardiac societies. This was ascribed due to the lack of consensus on a reference or standard method for quantification of HPR and unique cut-off values associated with increased clinical risk. Most importantly, studies showing improved clinical outcome by altering antiplatelet therapy based on platelet function assessment were lacking.

Clinical impact of altering antiplatelet therapy in patients with HPR

The Food and Drug Administration (FDA) announced a “Boxed Warning” on March 12, 2010, about the diminished effectiveness of clopidogrel in patients with an impaired ability to convert the drug into its active form (71). Although this warning focussed mainly on a decreased availability of the active metabolite in poor metabolisers of clopidogrel caused by genetic polymorphisms (CYP2C19 LOF) this statement raised much interest among clinicians as to whether they should perform routine testing either for genetic variants of the CYP2C19 LOF allele and/or for overall effectiveness for inhibition of platelet activity in patients being treated with clopidogrel. Furthermore, uncertainty was raised by the recommendation ‘the use of other anti-platelet medications or alternative dosing strategies for clopidogrel should be considered’, since prospective randomised clinical trials showing an improved clinical outcome by altered treatment regimens in patients with HPR irrespective of the underlying mechanism are lacking. So far, results from two major randomised prospective double-blind trials evaluating alternate treatment algorithms in patients with HPR on clopidogrel after PCI are available.

The GRAVITAS (Gauging Responsiveness with A VerifyNow assay—Impact on Thrombosis And Safety) study randomised 2,214 patients with stable coronary artery disease or non-ST-elevation acute coronary syndromes and HPR (≥230 PRU by VerifyNow P2Y12 test) assessed 12 to 24 h after PCI with one or more drug-eluting stent(s) (DES) to either high-dose clopidogrel (additional 600-mg bolus dose, 150 mg daily thereafter) or to standard-dose clopidogrel (no additional loading dose, 75 mg OD) (72). Patients were followed up to six months after PCI. Assessments of platelet reactivity were repeated during the course of the study at 30 days and six months after randomisation in a double-blind fashion. The use of high-dose clopidogrel compared with standard-dose clopidogrel did not reduce the incidence of the primary endpoint which was the combined incidence of death from cardiovascular causes, non-fatal MI, or ST. The primary endpoint occurred in each treatment arm in 25 patients corresponding to an equal incidence of 2.3 % in both strata (p=0.97; Figure 3). The rate of severe or moderate bleeding was not increased in patients randomised to high-dose clopidogrel. Persistent HPR at 30 days was observed in 62% of patients on standard dose vs. 40% of patients treated with high dose clopidogrel (p<0.001) which indicates that the additional antiplatelet effect of the increased dosing regimen of clopidogrel was only moderate. Besides the limited gain in additional antiplatelet efficacy by doubling the maintenance dose of clopidogrel, it should be considered that HPR resolved in 40% of subjects in the control arm already by day 30 after PCI. Moreover, a post hoc analysis of the results suggested that a lower than the pre-specified cut-off for HPR (208 instead of 230 P2Y12 reaction units) was associated with a lower risk for cardiovascular events and might have been a more appropriate cut-off for HPR. It should be considered that the observed clinical event rate in GRAVITAS was less than half of the expected six-month primary event rate of 5.0% which was assumed for statistical power calculation. The assumed event rate was derived from three previous studies (73-75): The study by Leon et al. was historical to some extent, because it enrolled patients in 1996, used Palmaz–Schatz coronary stents and compared three antithrombotic regimens namely aspirin alone, aspirin plus ticagrelor or aspirin plus ticlopidine 250 mg BID (73). The study by Buonamici et al. comprised a cohort with a much higher clinical risk profile, because patients were considered eligible for the study irrespective of clinical presentation or coronary anatomy (74). Thus, high proportions of patients with ACS and ST-segment elevation acute myocardial infarction (AMI) as well as patients with left main disease, chronic total occlusions, bifurcation lesions, or diffuse disease were included.

Patients undergoing elective PCI with at least one DES and HPR (VerifyNow P2Y12 reaction units [PRU] ≥208) were candidates for enrolment into TRIGGER-PCI (Testing platelet Reactivity In patients underGoing elective stent placement on clopidogrel to Guide alternative thErapy with pRasugrel), a multi-center, double-blind, randomised controlled trial (76). Platelet reactivity
was assessed 2-7 h after the first MD of clopidogrel 75 mg on the day after PCI following loading with clopidogrel 600 mg and patients with HPR were randomised to either prasugrel 10 mg OD or clopidogrel 75 mg OD. Primary endpoint was the incidence of cardiac death and MI during six-month follow-up. The primary safety endpoint was the six-month incidence of TIMI major bleeding. The study was powered assuming a 50% relative risk reduction with 100 primary endpoint events expected to occur in 2,150 patients randomised. TRIGGER-PCI was terminated prematurely for futility by the steering committee after randomisation of 423 patients because of a lower than expected incidence of the primary endpoint. The primary efficacy endpoint occurred in none of the patients on prasugrel versus one patient on clopidogrel. The primary safety endpoint of non–CABG TIMI major bleeding at six months occurred in three patients (1.4%) on prasugrel versus one (0.5%) on clopidogrel. Blinded platelet reactivity on study drug was assessed at three and six months. Analysis of the platelet function data showed, that in patients assigned to prasugrel, PRU decreased from 245 (225 to 273) (median [interquartile range]) at baseline to 80 (42 to 124) at three months, whereas in patients receiving clopidogrel, PRU decreased from 249 (225 to 277) to 241 (194 to 275) (p<0.001 vs. prasugrel). Thus, the antplatelet effect of prasugrel in patients with HPR on clopidogrel was very prominent. HPR was still present in 70.4% of patients on clopidogrel at three months, while only 5.9% of prasugrel treated exhibit HPR at that time point (p<0.001; Figure 4). Similar rates were observed at six months. Thus, TRIGGER-PCI demonstrated that HPR can be reliably corrected by switching from clopidogrel to prasugrel, but failed to demonstrate an improvement in clinical outcomes by switching patients with HPR on clopidogrel after elective PCI with DES implantation to the more potent P2Y12 inhibitor prasugrel. The study, however, questions if in low-risk patients, such as those enrolled in TRIGGER-PCI, testing platelet function for consideration of more intensive antiplatelet therapy is warranted, especially given the potentially increased risk of bleeding.

These findings are corroborated by a first analysis of the ADAPT-DES (Assessment of Dual AntiPlatelet Therapy with Drug-Eluting Stents) registry which aims to investigate the relationship between platelet responsiveness and definite or probable stent thrombosis after DES implantation without major procedural complications (77). Platelet function was assessed using the VerifyNow assay after clopidogrel (either loading or chronic therapy) and loading with aspirin and an adequate washout period if glycoprotein (GP) IIb/IIIa inhibitors were used peri-procedural. A first analysis of the 30-day incidence of ST in 8,439 patients overall showed that the absolute and relative levels of platelet inhibition by ADP antagonists as assessed by the VerifyNow P2Y12 test are powerful independent predictors of stent thrombosis (hazard ratio [HR] for P2Y12 PRU ≥230: 2.75; 95% CI 1.35-5.60; p=0.005). The large number of patients enrolled enabled subgroup analysis for patients with ACS (HR for P2Y12 PRU ≥230: adjusted for diabetes vs no diabetes and stent length: 2.95, 95% CI: 1.29 - 6.77; p=0.01; n=4,347) and non-ACS (adjusted HR for P2Y12 PRU ≥230: 2.02, 95% CI: 0.46 - 8.74; p=0.35; n=4,092). Thirty (ACS) and nine ST (non-ACS) were recorded, respectively. The very low ST rate in patients with stable coronary artery disease, together with the limited prognostic accuracy of platelet function testing in this setting suggests that assessing antiplatelet response in patients without ACS undergoing PCI is unlikely to provide incremental clinical utility. In contrast, assessment of platelet responsiveness to ADP antagonist loading might be useful to predict 30-day ST in patients with ACS.

A very recent meta-analysis aimed to analyse efficacy and safety of various approaches to intensify antiplatelet therapy based upon the verification of HPR on standard-dose clopidogrel. Only ADP-specific platelet function assays (VerifyNow P2Y12 assay, ADP-stimulated aggregometry, VASP assay, conventional light transmission or multiple electrode impedance aggregometry with ADP as stimulant) were considered appropriate for assessment of HPR (78). A literature research considered only prospective, randomised trials reporting clinical outcome of patients for this meta-analysis. Cardiovascular death, non-fatal MI and definite/probable ST were evaluated as efficacy outcome, while major bleeding events were recorded as safety endpoint.

Figure 3: Cumulative Kaplan-Meier estimates of the time to the first occurrence of the primary endpoint in patients with HPR receiving high- or standard-dose clopidogrel in GRAVITAS (72) (modified with permission from Dr. Price, slide from presentation at AHA Congress 2010).
Studies investigating short-term intensified antiplatelet regimens using either GP IIb/IIIa inhibitors or repetitive bolus dosing of clopidogrel and chronic intensified treatment algorithms with either additional bolus doses plus increased maintenance dosing of clopidogrel or switching to prasugrel were included (Table 1). Ten studies comprising 4,213 patients were identified. There were substantial differences between the studies regarding clinical presentation of the patients enrolled (ACS vs. elective PCI). The meta-analysis showed that in patients with HPR intensified antiplatelet therapy compared to standard antiplatelet therapy with clopidogrel reduced the rate of thrombotic events without increasing risk of major bleeding. A significant reduction in cardiovascular mortality (OR 0.38, 95%CI 0.18–0.78, p=0.008), in definite/probable ST (OR 0.38, 95%CI 0.19–0.75, p=0.006) and in non-fatal MI (OR 0.47, 95%CI 0.28–0.78, p=0.008) was observed. There was no difference in the incidence of major bleeding events between intensified and standard antiplatelet therapy (p=0.44). A meta-regression analysis indicated that the net benefit obtained by intensified antiplatelet regimens in patients with HPR might be greater in patients at higher risk of ST but not on the clinical presentation (ACS vs. elective PCI in stable angina). However, it should be considered that the database for this meta-analysis is limited (n=4,213 patients in total) with only one third of patients undergoing PCI in ACS being enrolled. Moreover, four out of 10 studies randomised fewer than 200 patients, and four different intervention strategies to intensify antiplatelet therapy in patients with HPR were used in these 10 studies: peri-procedural GP IIb/IIIa inhibitor infusion, a maximum of three repeated loading doses of clopidogrel 600 mg, increased maintenance doses of clopidogrel, and switching from clopidogrel to prasugrel. These limitations should be considered before drawing definite conclusions from this meta-analysis.

Two recent studies randomised patients after PCI either to personalised therapy after monitoring antiplatelet response or to conventional dosing. The Thrombocyte Activity Reassessment and GEnoTyping for PCI (TARGET-PCI) study (ClinicalTrials.gov Identifier: NCT01177592) was a prospective, single-centre, randomised trial which planned to enroll 1,500 subjects requiring PCI. The study randomised eligible subjects to either a guided antiplatelet therapy arm (n=750) or a standard therapy arm (n=750) with a clinical follow-up for one year. Patients in the guided therapy group underwent laboratory testing with antiplatelet adjustment if they have a measurement of ≥ 230 PRU by VerifyNow P2Y12 testing. They were reloaded with 60 mg prasugrel and continued on standard maintenance dosing of prasugrel. Similarly, clopidogrel-naive subjects that are considered CYP2C19*2 carriers (Verigene CYP2C19 genotyping assay) were also reloaded with 60 mg prasugrel and received standard maintenance dosing thereafter. Patients randomised to the control arm remained on 75 mg clopidogrel arm throughout the study. Unfortunately, the study was terminated prematurely in November 2011.

The Double Randomisation of a Monitoring Adjusted Antiplatelet Treatment Versus a Conventional Antiplatelet Treatment for DES Implantation, and Interruption Versus Continuation of Double Antiplatelet Therapy (ARCTIC) study (ClinicalTrials.gov Identifier: NCT00827411) completed enrolment of 2,500 patients. ARCTIC randomised patients after DES-PCI either to a monitoring arm comprising dose adjustment of aspirin and clopidogrel (or switch to prasugrel) based on antiplatelet response assessed by using the VerifyNow system or to a conventional strategy with fixed dose regimens of both aspirin (75 mg OD) and clopidogrel (75 mg OD). Two to four weeks after PCI, platelet function was reassessed in the monitoring arm enabling a second adjustment (either increase or decrease in intensity) of antiplatelet therapy. ARCTIC showed no significant improvements in clinical outcomes (composite of death, MI, ST, stroke, or urgent revascularisation) with platelet function monitoring and treatment adjustment, as compared with standard antiplatelet therapy without monitoring at one year after stent implantation (96).

Does clinical presentation of the patient strike out platelet function assessment?

In ACS patients, the one-size-fits-all concept by using more intensified antiplatelet regimens without personalising treatment strategy based upon platelet function data seems attractive. Intensified inhibition of the P2Y12 receptor has shown a reduction of cardiovascular ischaemic events in two large-scale clinical trials which randomised ACS patients to intensified antiplatelet regimens vs. standard dose clopidogrel without any platelet function testing (53, 54).

Treatment of ACS patients undergoing PCI with prasugrel compared with standard-dose clopidogrel reduced the combined primary endpoint of death from cardiovascular causes, non-fatal MI, or non-fatal stroke in TRITON-TIMI 38 by 2.2% (53). Patients enrolled into the PLATO study received either the reversible direct-acting P2Y12 inhibitor ticagrelor or clopidogrel. Treatment with ticagrelor as compared with clopidogrel reduced the rate of the composite endpoint of death from vascular causes, MI, or
stroke by 1.9% in ACS patients (54). However, a significant increase in non-CABG-related TIMI major bleedings by 0.5 to 0.6 absolute percentages was observed in both studies in patients receiving the intensified antiplatelet regimen.

The CURRENT – OASIS 7 trial used a 2 x 2 factorial design and randomised patients to high- and low-dose aspirin and double dose clopidogrel (600 mg loading dose followed by 150 mg daily for seven days) versus the approved dose regimen of clopidogrel (300 mg loading dose of clopidogrel followed by 75 mg OD) (79). The trial was negative showing no significant benefit of higher dose clopidogrel. Nevertheless, in the subgroup analysis of patients undergoing PCI, a seven-day double-dose clopidogrel regimen was associated with a reduction in 30-day cardiovascular events and ST compared with the standard dose (80).

Table 1: Characteristics of prospective randomised studies investigating efficacy and safety of intensified antiplatelet therapy based upon ADP-specific platelet function testing (modified from Aradi et al. (78)).

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of pts. (tailored / control)</th>
<th>Clinical presentation</th>
<th>Platelet function assay</th>
<th>Cut-off for HPR</th>
<th>Proportion of pts. with HPR (%)</th>
<th>Intensified treatment strategy</th>
<th>Duration of intervention</th>
<th>Follow-up</th>
<th>Primary endpoint</th>
<th>Incidence of primary endpoint (tailored / control)</th>
<th>Primary endpoint reached p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonello et al. (88)</td>
<td>78 / 84</td>
<td>52% stable angina 48% NSTEMI 0% STEMI</td>
<td>VASP</td>
<td>VASP-PRI &gt;50%</td>
<td>52%</td>
<td>Repeated LD of clopidogrel 600 mg (up to 3 times)</td>
<td>One week</td>
<td>30 days</td>
<td>CV death, definite ST, recurrent ACS, revascularisation</td>
<td>0% vs. 10%</td>
<td>Yes P=0.007</td>
</tr>
<tr>
<td>Bonello et al. (89)</td>
<td>215 / 214</td>
<td>48% stable angina 52% ACS 0% STEMI</td>
<td>VASP</td>
<td>VASP-PRI &gt;50%</td>
<td>45%</td>
<td>Repeated LD of clopidogrel 600 mg (up to 3 times)</td>
<td>One week</td>
<td>30 days</td>
<td>Early definite ST</td>
<td>0.5% vs. 4.7%</td>
<td>Yes P=0.010</td>
</tr>
<tr>
<td>Valgimigli et al. (90)</td>
<td>79 / 91</td>
<td>47% stable angina 53% ACS 0% STEMI</td>
<td>VerifyNow P2Y12</td>
<td>&lt;40% inhibition</td>
<td>48%</td>
<td>Tirofiban 25 µg/kg bolus + 0.15 µg/kg/min. infusion</td>
<td>14 – 24 h</td>
<td>30 days</td>
<td>Peri-procedural MI (&lt;48h)</td>
<td>20.4% vs. 35.1%</td>
<td>Yes P=0.009</td>
</tr>
<tr>
<td>Cuisset et al. (91)</td>
<td>74 / 75</td>
<td>100% stable angina 0% ACS 0% STEMI</td>
<td>LTA 10 µM ADP</td>
<td>&gt;70% max. aggregation</td>
<td>23%</td>
<td>Abciximab 0.25 mg/kg bolus + 0.125 µg/kg/min. infusion</td>
<td>14 – 24 h</td>
<td>30 days</td>
<td>Death, Def./prob. ST, recurrent ACS</td>
<td>19% vs. 40%</td>
<td>Yes P=0.006</td>
</tr>
<tr>
<td>Aradi et al. (92)</td>
<td>36 / 38</td>
<td>100% stable angina 0% NSTEMI 0% STEMI</td>
<td>LTA 5 µM ADP</td>
<td>&gt;34% max. aggregation</td>
<td>38%</td>
<td>600 mg LD + 150 mg MD clopidogrel</td>
<td>1 month</td>
<td>12 months</td>
<td>CV death, MI, TVR</td>
<td>3.1% vs. 24.6%</td>
<td>Yes P=0.010</td>
</tr>
<tr>
<td>Ari et al. (93)</td>
<td>47 / 47</td>
<td>100% stable angina 0% NSTEMI 0% STEMI</td>
<td>VerifyNow P2Y12</td>
<td>&lt;40% inhibition</td>
<td>49%</td>
<td>150 mg MD clopidogrel</td>
<td>1 month</td>
<td>6 months</td>
<td>CV death, MI, ST, TVR or recurrent ACS</td>
<td>4.3% vs. 17%</td>
<td>Yes P=0.045</td>
</tr>
<tr>
<td>Hazarbasanov et al. (94)</td>
<td>97 / 95</td>
<td>43% stable angina 33% NSTEMI 24% STEMI</td>
<td>Multiplate 6.4 µM ADP</td>
<td>&gt;46 AU</td>
<td>19%</td>
<td>600 mg LD + 150 mg MD clopidogrel</td>
<td>1 month</td>
<td>6 months</td>
<td>CV death, MI, ST or ischaemic stroke</td>
<td>0% vs. 5.3%</td>
<td>Yes P=0.022</td>
</tr>
</tbody>
</table>
High on-treatment platelet reactivity

The results of GRAVITAS, TRIGGER-PCI, ADAPT-DES and ARCTIC have major impact on the concept of personalised antiplatelet therapy. It seems that outcomes in elective PCI have improved within recent years most likely due to improvements in stent technology and operator skills. Recent studies with new-generation DES show substantially better outcomes than with first generation DES (81-83). It seems that these patients can tolerate HPR without increased risk for ischaemic events after DES implantation if PCI was not accompanied by major procedural complications, procedure-related infarctions, or peri-interventional bleeding.

On the other hand, patients with ACS treated with the more effective new platelet inhibitors prasugrel or ticagrelor administered in standard doses have a clear benefit regarding ischaemic events (53, 54). The reduced incidence of ischaemic endpoints is associated with an increased risk of non-CABG (coronary artery bypass graft) major bleeding in these studies. Although the benefit regarding ischaemic events is not outweighed by the increased risk for bleeding on these new drugs, it seems that there is still room for optimisation of antplatelet therapy to increase the net clinical benefit. A recent observational study assessed platelet reactivity after prasugrel loading (60 mg) in ACS patients undergoing PCI (84). Despite the use of prasugrel, HPR according to the cut-off of the VASP assay was reported in 25% of patients and more thrombotic events were observed in these patients within 30-days follow-up.

Thus, future randomised trials are warranted to optimise the risk-benefit ratio of intensified antplatelet therapy. Procedural characteristics impacting on risk of ST such as multi-lesion and/or complex lesion interventions, and peri-interventional complications should be considered in future clinical trials. Data on increased risk of bleeding associated with very low levels of platelet reactivity are sparse and are from observational studies only (85, 86). Therefore, these studies should not only aim to improve platelet inhibition but also to reduce risk of bleeding answering the question of a “therapeutic window” regarding antplatelet therapy.

Conflicts of interest
D.T. reported receiving consulting fees or paid advisory board fees and lecture fees from Eli Lilly, Daiichi Sankyo and AstraZeneca and lecture fees from Boehringer Ingelheim KG. S.D.K. has received lecture fees from AstraZeneca and Eli Lilly. W.H. reported serving on a consultant/advisory board for Sanofi-Aventis. F.-J.N. declared no personal conflict of interest. F.-J.N.‘s institution received speaker honoraria, consultancy fees and research grants from Eli Lilly, Daiichi Sankyo, Sanofi-Aventis, Bristol, Novartis, Roche, Boston Scientific, Cordis, Biotronik, and Medtronic.

Future perspectives

Table 1: Continued

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of pts. (tailored / control)</th>
<th>Clinical presentation</th>
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<th>Incidence of primary endpoint (tailored / control)</th>
<th>Primary endpoint reached p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang et al. (95)</td>
<td>150 / 156</td>
<td>80% stable angina 20% NSTEMI 0% STEMI</td>
<td>VASP</td>
<td>VASP-PRI &gt;50%</td>
<td>57%</td>
<td>Stepwise increase in clopidogrel MD up to 375 mg acc. to VASP-PRI</td>
<td>11 months (from the 1st month)</td>
<td>12 months</td>
<td>CV death, definite ST, recurrent ACS and revascularisation</td>
<td>9.3% vs. 19.2%</td>
<td>Yes P=0.008</td>
</tr>
<tr>
<td>Price et al. (72)</td>
<td>1109 / 1105</td>
<td>60% stable angina 10% NSTEMI 29% unstable angina 0.4% STEMI</td>
<td>VerifyNow P2Y12</td>
<td>&gt;230 PRU</td>
<td>41%</td>
<td>600 mg LD + 150 mg clopidogrel</td>
<td>6 months</td>
<td>6 months</td>
<td>CV death, non-fatal MI, definite / probable ST</td>
<td>2.3% vs. 2.3%</td>
<td>No P=0.970</td>
</tr>
<tr>
<td>Trenk et al. (76)</td>
<td>212 / 211</td>
<td>100% stable angina 0% NSTEMI 0% STEMI</td>
<td>VerifyNow P2Y12</td>
<td>&gt;208 PRU</td>
<td>19%</td>
<td>60 mg LD + 10 mg prasugrel</td>
<td>6 months</td>
<td>6 months</td>
<td>CV death, MI</td>
<td>0% vs. 0.5%</td>
<td>No NE</td>
</tr>
</tbody>
</table>

ACS: acute coronary syndrome; CV: cardiovascular; HPR: high on-treatment platelet reactivity; LD: loading dose; MD: maintenance dose; MI: myocardial infarction; NE: not evaluated due to insufficient data; NSTEMI: non-ST segment elevation myocardial infarction; STEMI: ST-segment elevation myocardial infarction; TVR: target vessel revascularisation.
References


Trenk et al. HPR and P2Y12 antagonists in clinical trials