

# Impact of Genetic Polymorphisms and Drug–Drug Interactions on Clopidogrel and Prasugrel Response Variability

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**Abstract:** Thienopyridine antiaggregating platelet agents (clopidogrel and prasugrel) act as irreversible P2Y<sub>12</sub> receptor inhibitors. They are used with aspirin to prevent thrombotic complications after an acute coronary syndrome or percutaneous coronary intervention. A large interindividual variability in response to clopidogrel and to a lesser extent to prasugrel is observed and may be related to their metabolism. Clopidogrel and prasugrel are indeed prodrugs converted into their respective active metabolites by several cytochromes P450 (CYPs). Besides clopidogrel inactivation (85%) by esterases to the carboxylic acid, clopidogrel is metabolized by CYPs to 2-oxo-clopidogrel (15%) and further metabolized to an unstable but potent platelet-aggregating inhibitor. Prasugrel is more potent than clopidogrel with a better bioavailability and lower pharmacodynamic variability. Prasugrel is completely converted by esterases to an intermediate oxo-metabolite (R-95913) further bioactivated by CYPs. Numerous clinical studies have shown the influence of CYP2C19 polymorphism on clopidogrel antiplatelet activity. Moreover, unwanted drug–drug pharmacokinetic interactions influencing CYP2C19 activity and clopidogrel bioactivation such as with proton pump inhibitors remain a matter of intense controversy. Several studies have also demonstrated that CYP3A4/5 and CYP1A2 are important in clopidogrel bioactivation and should also be considered as potential targets for unwanted drug–drug interactions. Prasugrel bioactivation is mainly related to CYP3A4 and 2B6 activity and therefore the question of the effect of drug–drug interaction on its activity is open. The purpose of this review is to critically examine the current literature evaluating the influence of genetic and environmental factors such as unwanted drug–drug interaction affecting clopidogrel and prasugrel antiplatelet activity.

**Keywords:** Clopidogrel, cytochrome P450, interaction, pharmacokinetic, pharmacodynamic, pharmacogenetic, platelet, prasugrel.

## INTRODUCTION

Platelets play a key role in thrombus formation that occurs after atherosclerotic plaque rupture in acute coronary syndrome (ACS) or in a stent after percutaneous coronary intervention (PCI). Antiplatelet drugs like clopidogrel and prasugrel are second- and third-generation thienopyridines respectively that irreversibly inhibit the P2Y<sub>12</sub>-adenosine diphosphate (ADP) receptor. Clopidogrel is widely used to reduce recurrence of atherothrombotic events in patients with recent myocardial infarction (MI), stroke, established peripheral arterial disease, or ACS [1, 2]. It was the second drug most sold worldwide (\$8.6 billion) in 2008 [3].

A high interindividual variability of the biological response to clopidogrel is observed in cardiovascular patients. Some patients treated with clopidogrel have test values close to untreated subjects. These patients are usually deemed “resistant” or “poor responders” to clopidogrel. There are different types of biological assays to assess the biological responsiveness to clopidogrel and the prevalence of poor responders is variable. The variable specificity of the assays regarding clopidogrel target accounts in part for the variability of the prevalence of poor responders. Indeed, it has been reported that 6–25% of patients are resistant to clopidogrel with various assays [4]. Light transmission aggregometry was considered as the gold standard for the evaluation of antiplatelet drug responsiveness until recently. However, the lack of standardization and the poor specificity in assessing P2Y<sub>12</sub> inhibition of this procedure belong to the drawbacks of this assay and new, more specific assays may be more suitable to monitor clopidogrel potency [5]. The clinical relevance of clopidogrel poor responsiveness was recently assessed in a meta-analysis: 15 studies were assessed, including 3960 patients, 25% of whom were considered as clopidogrel poor responders. The global relative risk (RR) for recurrent ischemic events in clopidogrel poor responders was 3.5 ([2.4–5.2],  $p < 0.0001$ ) [6]. The etiology of the biologically poor responsiveness to

clopidogrel is probably multifactorial: Bonello–Palot *et al.* showed in a prospective study in 73 patients that high body mass index (BMI) ( $p = 0.01$ ), diabetes mellitus ( $p = 0.03$ ), ACS ( $p = 0.02$ ), and CYP2C19\*2 variant allele ( $p = 0.04$ ) were associated with high on-treatment platelet reactivity after a 600-mg intake of clopidogrel [7]. These data were confirmed by a recent study that identified predictive factors of insufficient response to antiplatelet treatment in 760 patients undergoing elective coronary stent implantation after a 600 mg intake of clopidogrel. The CYP2C19\*2 carrier status (OR: 2.74; 95% CI: 1.93–3.90), age (OR: 1.03; 95% CI: 1.01–1.05), diabetes mellitus (OR: 1.75; 95% CI: 1.19–2.56), and high body mass index (OR: 1.06; 95% CI: 1.02–1.11) were the main predictors for poor response to clopidogrel [8]. The CYPs necessary for the activation of the drug, and their level of expression and activity, seem to play a crucial role. Indeed, the FDA released a warning in March 2010 to inform healthcare professionals that: “effectiveness of Plavix depends on activation to an active metabolite by the cytochrome P450 (CYP) system, principally CYP2C19; poor metabolizers treated with Plavix at recommended doses exhibit higher cardiovascular event rates following ACS or PCI than patients with normal CYP2C19 function; tests are available to identify a patient’s CYP2C19 genotype and can be used as an aid in determining therapeutic strategy; consider alternative treatment or treatment strategies in patients identified as CYP2C19 poor metabolizers” [9].

Recently, attention was drawn on patients on the other end of the biological clopidogrel-response distribution curve (i.e., “high responders”). Indeed, these patients may be at higher risk of bleeding, and this association may be driven by an increased activity of CYPs, possibly through a genetic variant of the CYP2C19 gene [10, 11].

Along with CYPs activity, other causes of the variability of response to clopidogrel may include genetic and environmental factors affecting the absorption (P-glycoprotein), the activity of carboxylesterases and its binding to the platelet P2Y<sub>12</sub> receptor.

Prasugrel, a novel thienopyridine recently approved, has a lower biological interindividual variability than clopidogrel [12]. It has a better bioavailability and is metabolized to its pharmacologically active metabolite by other CYPs [13, 14]. Indeed, the different

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CYPs involved in the metabolism of the two molecules could explain the differences in the interactions observed with clopidogrel or prasugrel, such as the recently discussed interaction with proton pump inhibitors (PPIs) [15].

The aim of this review was to describe the factors affecting the interindividual variability in response to the antiplatelet drugs clopidogrel and prasugrel with focus on pharmacogenetic factors and the clinically pertinent drug–drug interactions.

### THIENOPYRIDINES IN PATIENTS WITH CARDIOVASCULAR EVENTS

Ticlopidine was the first thienopyridine that was approved in the market, but the risk of neutropenia (1% of patients) and thrombotic thrombocytopenic purpura (0.02%) limited its use. Clopidogrel, with a better safety profile, has now largely supplanted ticlopidine. Although the CAPRIE study showed a small, but significant advantage of clopidogrel over aspirin in the secondary prevention of cardiovascular patients, thienopyridines are usually prescribed in combination with aspirin [16].

The benefit of the dual-therapy aspirin, clopidogrel, was evidenced in several large-scale trials that included cardiovascular patients within the full range of ACS (unstable angina, non-ST elevation myocardial infarction, and ST-elevation myocardial infarction) [17]. The clinical development of the third generation thienopyridine prasugrel was evaluated in association with aspirin.

The phase II randomized study JUMBO-TIMI 26 (Joint Utilization of Medications to Block Platelets Optimally-Thrombolysis in Myocardial Infarction 26) has evaluated the safety profile of clopidogrel and prasugrel in 904 patients before a PCI and were randomized in four groups (clopidogrel 300mg LD or prasugrel 60mg LD +10mg/day or 60mg LD +15mg/day or prasugrel 40mg LD +7.5mg/day). This study showed that the rate of minor bleedings was higher in patients with high doses of prasugrel and a dose-effect relation was demonstrated [18].

The double-blind randomized crossover study PRINCIPLE TIMI 44 (Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation–Thrombolysis in Myocardial Infarction 44) realised in 201 subjects with a planned PCI, showed that prasugrel allowed a better platelet aggregation inhibition than clopidogrel at both loading and maintenance doses in patients with stable coronary disease (LD: 78.4% vs. 31.8%; NNT=2.3 and MD:61.3% vs. 46.1%; NNT=6.6) [19].

The phase III study TRITON-TIMI 38 recently evaluated prasugrel in patients with ACS [20]. Although no difference was observed in terms of mortality in both the arms, prasugrel was associated with a relative decrease in the occurrence of ischemic cardiovascular events by 19% (odds ratio of 0.81, 95% confidence interval: 0.73–0.90). Most of the benefit was evidenced very early (<3 days) and maintained during the whole period of follow-up (6–15 months). However, an increased risk of bleeding, including fatal hemorrhage was observed with prasugrel treatment.

Contrary to situations of acute coronary artery injury, aspirin–thienopyridine combination therapy has been disappointing, so far, in other clinical settings. Both the MATCH trial that included patients with recent transient ischemic attack (TIA) or ischemic stroke and the CHARISMA trial that included cardiovascular patients at high risk, did not show any supplementary reduction in the cardiovascular events' composite endpoint and showed an increased risk of hemorrhagic complications, when compared to monotherapy (clopidogrel or aspirin alone). The ACTIVE-W trial showed the superiority of anticoagulation as compared to aspirin/clopidogrel dual therapy for the prevention of recurrence of ischemic stroke in patients with atrial fibrillation [21]. However, this dual antiplatelet therapy appeared to be superior than aspirin alone in patients with atrial fibrillation, who were not suitable for anticoagulation therapy with a significant 11% reduction in vascular events in those receiv-

ing clopidogrel as compared with placebo, but there was a 57% higher incidence of major and severe bleeding that have been reported [22]. To our knowledge, there is no trial evaluating prasugrel in stable cardiovascular patients. The SWitching AntiPlatelet Study (SWAP) study investigated the biological effect of the treatment switch from clopidogrel to prasugrel in 100 patients with acute coronary syndrome. The results showed that switching from clopidogrel to prasugrel is associated with a reduction in platelet function by one week when prasugrel is administered at maintenance dose and by two hours with the administration of prasugrel loading dose [23].

Other studies are currently in progress to compare the efficacy of clopidogrel and prasugrel in acute settings: Targeted platelet Inhibition to Clarify the Optimal strategy to medically managed Acute Coronary Syndromes, NCT00699998 (TRILOGY-ACS) is designed to test the hypothesis that the combination of aspirin and prasugrel is superior than the combination of aspirin and clopidogrel in the treatment of medically managed patients enrolled within 10 days after ACS. The Optimizing Antiplatelet Therapy in Diabetes Mellitus, NCT00642174 (OPTIMUS-3) is also in progress to compare the pharmacodynamic response to clopidogrel and prasugrel in patients with diabetes and coronary heart disease [24].

### PHARMACOLOGY OF CLOPIDOGREL

Clopidogrel is a prodrug that requires to be metabolized to an active metabolite by CYPs to produce its antiaggregating effect. Inhibition of ADP-induced platelet aggregation reaches a plateau after 5 days of standard-dose therapy (75 mg/d clopidogrel) [25]. After acute vessel injury, such as coronary stenting, the onset of clopidogrel action can be brought up to 2–5 h by the administration of a loading dose (300 or 600 mg) [26]. From a pharmacodynamical point of view, the clopidogrel active metabolite irreversibly inhibits the P2Y<sub>12</sub> receptor on platelets, leading to the maintenance of a high platelet cAMP level and to the non-activation of GPIIb/IIIa receptor, and consequently the inhibition of platelet aggregation. Due to the irreversible inhibition of the P2Y<sub>12</sub> receptor, recovery of platelet function occurs within 3–5 days [27] and the platelet function returns to a normal level approximately one week after the last dose of clopidogrel [28].

From a pharmacokinetic point of view, clopidogrel is rapidly absorbed after administration. Maximum plasma concentration (C<sub>max</sub>) of the clopidogrel active metabolite is obtained within the first hour with a C<sub>max</sub> of 160 ng/ml and an area under the curve (AUC) of 267 ng.h/ml after a loading dose of 600 mg. From the healthy volunteers who received a loading dose of 300 mg clopidogrel and 75 mg daily during 7 days, it was found that the maximal concentration was around 60 ng/ml [29]. As shown in 2006 by Taubert *et al.*, using a Caco-2 cell model, clopidogrel is transported by the efflux transporter P-glycoprotein, which is a key factor in the intestinal absorption of clopidogrel and this reduces its bioavailability [30]. Von Beckerath *et al.* showed that a dose of 900 mg clopidogrel did not increase the pharmacological effect as compared to 600 mg [31].

Clopidogrel is mainly metabolized to an inactive carboxylic acid derivative (SR 26334) generated through hydrolysis (85%). Only 15% of clopidogrel is metabolized to a pharmacologically active metabolite through a two-step oxidation process via CYPs, after the metabolization to an intermediate molecule: 2-oxo-clopidogrel (Fig. 1).

The metabolism of clopidogrel is still not totally understood, even though the CYP3A4, CYP2C19, and CYP1A2 seem to be the most implicated.

A study first demonstrated the main implication of the CYP1A subfamily in clopidogrel metabolism by measuring platelet aggregation after the induction of various CYPs isoforms in rat liver microsomes [32].

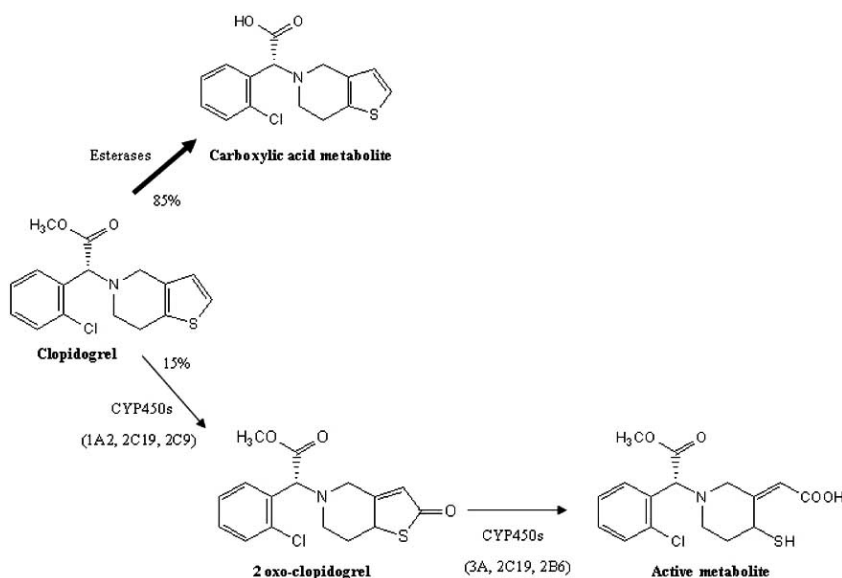


Fig. (1). Pathways of clopidogrel metabolism.

Farid *et al.* clearly established the role of CYP3A in a randomized controlled study in healthy subjects, where they demonstrated that ketoconazole, a potent CYP3A inhibitor, decreased the concentration of clopidogrel AM [33]. Another study identified CYP3A4 and CYP3A5 as the major CYPs involved in clopidogrel metabolism [34].

Lau *et al.* were among the first investigators demonstrating a drug-drug interaction that implicated clopidogrel. They showed that the addition of atorvastatin, (metabolized by CYP3A4) to clopidogrel, produced a dose-dependent reduction of the antiplatelet effect of clopidogrel in patients with a coronary stent. In another study, the same group showed an inverse correlation between platelet aggregation and CYP3A4 activity ( $p=0.003$ ). These findings support the implication of CYP3A4 in the clopidogrel metabolism [35, 36].

In a recent *in vitro* study, Kazui *et al.* assessed the CYPs involved in clopidogrel metabolism using human microsomes and supersomes. CYP2C19 and CYP1A2 were involved mainly in the first step of oxidation from clopidogrel to 2-oxo-clopidogrel (44.9% and 35.8% respectively), whereas CYP3A4, CYP2B6 and CYP2C19 were implicated in the active metabolite production by 39.8%, 32.9% and 20.6%, respectively. Globally, CYP2C19 was involved in the two oxidative steps of clopidogrel metabolism, whereas CYP3A4 only contributed to the second oxidative step [37]. About 40% of the total drug is excreted in urine and 35–60% in feces [38].

## INTERINDIVIDUAL VARIABILITY IN THE RESPONSE TO CLOPIDOGREL

### Absorption

Clopidogrel absorption is mediated by the efflux transporter P-glycoprotein (P-gp, MDR1). Using caco-2 cells, Taubert *et al.* showed in 2006, that clopidogrel is transported by the P-gp. They also investigated the influence of the C3435T (rs1045642) polymorphism of the ABCB1 gene coding for the MDR1 protein on plasma concentration of clopidogrel and its active metabolite. The genotype 3435TT was associated with a diminution of the concentration of the active metabolite of clopidogrel [30]. Simon *et al.* performed a pharmacogenetic study in myocardial infarction patients on clopidogrel and compared the clinical outcomes according to C3435T genotype. Individuals with the 3435TT genotype were at increased risk of recurrence of an ischemic events at one year

( $p=0.04$ ) [39]. The association between the 3435TT genotype and ischemic event in patients treated with clopidogrel was further supported by the data of the TRITON trial. In this latter analysis, TT homozygotes had a 72% increased risk of the primary endpoint compared with CT/CC individuals (HR 1.72, 95% CI 1.22, .44,  $p=0.002$ ) [40]. These *in vitro* and *in vivo* data suggest that a ABCB1 gene polymorphism plays a role in the interindividual variability of clopidogrel response.

## Metabolism

### Carboxylesterases Polymorphism

Carboxylesterases are important pharmacological determinants of drugs containing ester linkages such as clopidogrel and prasugrel. These antiplatelet agents are first hydrolyzed by esterases to an intermediate inactive metabolite (2-oxo) in the case of prasugrel and to an inactive carboxylic acid metabolite in the case of clopidogrel. CYPs are responsible for the further production of the active metabolites. In the case of clopidogrel, 85% of the drug is eliminated as an inactive carboxylic acid derivative.

The liver contains the highest carboxylesterase activity and expresses two major human carboxylesterases: HCE1 and HCE2. These enzymes exhibit a large interindividual variability in their expression and could be considered as a key step in the variability of the antiplatelet drugs response. HCE1 is found at particularly high concentrations in hepatic microsomes with levels nearly 50-fold higher than HCE2, based on quantitative immunoblotting data [41]. In addition to their expression in the liver, tissue-specific expression has been observed, HCE2 being expressed at relatively high levels in the small intestine, while HCE1 is not expressed in this tissue [42]. The involvement of the carboxylesterases HCE1 and HCE2 in aspirin and clopidogrel hydrolysis processes were assessed *in vitro*. It was demonstrated that liver microsomes hydrolyze aspirin and clopidogrel whereas intestinal microsomes hydrolyze aspirin only. The clopidogrel hydrolysis seems to be mainly mediated by HCE1 [43].

Like cytochromes P450, activity of carboxylesterases may be inhibited or induced by some drugs. In human primary hepatocytes, dexamethasone and phenobarbital were associated with a slight or moderate induction of HCE1 and HCE2 [44]. Moreover, *in vitro* data showed that carboxylesterases might be modulated by certain polycyclic aromatic hydrocarbons found in cigarette smoke [45]. Potential drug–drug interactions are therefore possible with clopidogrel

or prasugrel. Shi *et al.* showed in transfected cells with a cDNA construct encoding for HCE1 and in liver microsomes that the hydrolysis of oseltamivir, an antiinfluenza viral agent, was inhibited to as much as 90% in the presence of 50  $\mu$ M clopidogrel. This interaction decreased the therapeutic activity of oseltamivir [46].

However, to date, there is insufficient data to draw any conclusions regarding the impact of the interindividual variability of the carboxylesterases activity on the efficacy of antiplatelet drugs.

#### **CYP2C19 Polymorphism**

The impact of CYP2C19 genetic variant (CYP2C19\*1/\*2, rs4244285) on the biological response to clopidogrel was first described by Brandt *et al.* [47] followed by the publications of Hulot *et al.* [48] and Fontana *et al.* [49] who evaluated the association of the CYP2C19\*2 allele with platelet function tests in clopidogrel-treated healthy subjects. Several pharmacokinetic and clinical studies further investigated the impact of CYP2C19 polymorphism on clopidogrel pharmacokinetic, pharmacodynamic and on clinical outcomes.

A retrospective analysis in 74 healthy subjects genotyped for different CYPs and receiving a 300mg clopidogrel loading dose showed that subjects carrying the loss-of-function CYP2C19\*2 allele had a lower Cmax of clopidogrel active metabolite ( $p=0.02$ ), lower inhibition of platelet aggregation (0.003) and were more often defined as poor metabolizers ( $p=0.03$ ) than the non carriers [14].

In 2008, a study in 24 healthy subjects assessed the effect of CYP2C19 genotype on the plasma concentration of clopidogrel and its antiplatelet effect. They showed that plasma levels of clopidogrel were higher in poor metabolizers than in heterozygous extensive metabolizers or homozygous extensive metabolizers ( $p=0.008$ ). Poor metabolizers had a lower antiplatelet effect than heterozygous or homozygous extensive metabolizers ( $p<0.001$ ). However a limitation of this study is that the plasma concentration of the active metabolite of clopidogrel was not measured. Indeed, clopidogrel plasma concentration may be not representative of plasma concentration of the active metabolite [50].

In a recent genome-wide association study (Pharmacogenomics of Antiplatelet Intervention, PAPI study), the CYP2C19\*2 genotype accounts for 12% of the variation of clopidogrel response and addition of factors such as age, BMI and lipid levels almost doubles the variation of clopidogrel response that could be explained [51]. Thus, part of the variation of clopidogrel responsiveness is explained by the CYP2C19 variant, 10% by other known clinical factors and a major part by other poorly characterized factors, that include drug-drug interactions, body weight and possibly other genetic variants (ABCB1 for example). These data were further supported by a study in 760 cardiovascular patients; in this latter study, the CYP2C19\*2 genotype accounted for only 5.2% of the antiplatelet response of clopidogrel as assessed with ADP aggregation after a 600-mg loading dose [8]. This is consistent with the wide variation of clopidogrel responsiveness in both carriers and non-carriers of the CYP2C19 variant allele: a significant proportion of CYP2C19\*2 carriers have a low platelet response to ADP (adequate response to clopidogrel) while a significant proportion of patients with no mutated allele have a high platelet response to clopidogrel (clopidogrel resistance).

Assessing the impact of CYP2C19\*2 and other loss of function genetic variants (\*3) on clinical events was the next step. However, the influence and magnitude of risk of the CYP2C19 genotype have been inconsistent among studies. Two meta-analysis tried to summarize these data. A first analysis gathered data on more than 8000 cardiovascular patients treated with clopidogrel out of 7 studies and showed that the CYP2C19\*2 allele was associated with major adverse cardiovascular events (MACE) (relative risk: 1.96, 95%CI: 1.14–3.37,  $p=0.02$ ) [52]. The second analysis gathered data on more than 11000 patients (23 studies) and showed that the CYP2C19\*2

allele was independently associated with an increase in the risk for MACE compared with noncarriers (9.7% vs. 7.8%; OR: 1.29; 95% CI: 1.12 to 1.49;  $p<0.001$ ), with an excess of mortality (1.8% vs. 1.0%; OR: 1.79; 95% CI: 1.10 to 2.91;  $p<0.019$ ;  $n=6225$ ) and of stent thrombosis (2.9% vs. 0.9%; OR: 3.45; 95% CI: 2.14 to 5.57;  $p<0.001$ ;  $n=4905$ ). Although reduced CYP2C19 activity only moderately shifts the distribution of response to clopidogrel in CYP2C19\*2 carriers, there is growing evidence that there could be a threshold level of platelet reactivity at which the risk of arterial thrombotic events substantially increases, which might explain why such a limited shift in population response could have an effect on the risk of recurrent arterial thrombosis [53].

Finally, since reduced CYP2C19 activity is associated with ischemic cardiovascular events in clopidogrel-treated patients, an increased activity conferred by other genetic variants such as the CYP2C19\*17 allele (rs12248560) may be associated with an increased response to clopidogrel and bleeding events compared to noncarriers of this allele. This was indeed demonstrated in a recent study involving 1524 cardiovascular patients [54].

#### **CYP3A4 and 3A5 Polymorphism**

Results on the influence of an intronic polymorphism of the CYP3A4 gene (IVS10 + 12A) are conflicting. This genetic variant was found to be associated with clopidogrel responsiveness in 82 cardiovascular patients [55], while results on 94 healthy subjects did not show any association [49].

In 2008, Kim *et al.* demonstrated that the CYP3A5\*3 genetic variant was not associated with a modulation of the effect of clopidogrel on platelet aggregation in 28 healthy subjects. Pharmacokinetics (clopidogrel and inactive metabolite concentrations in plasma) and pharmacodynamics (platelet aggregation) parameters of clopidogrel were shown to be similar in the three groups (CYP3A5\*1/\*1, CYP3A5\*1/\*3, and CYP3A5\*3/\*3) [56]. These latter results were confirmed in 162 healthy subjects and the CYP3A5\*3 polymorphism was not associated with the occurrence of clinical events in the TRITON TIMI 38 trial [57].

The main drawback of pharmacogenetic studies involving CYP3A enzymes is the poor correlation between the polymorphisms of these enzymes and their activity. Indeed, Oneda *et al.* demonstrated a weak correlation between CYP3A phenotypes (with midazolam clearance test) and CYP3A genotypes (CYP3A4, CYP3A5, and CYP3A7 variants). In a meta-analysis from seven clinical trials, the correlation between CYP3A4/5 alleles and midazolam disposition was assessed. There was no difference in midazolam disposition between different genotypes, haplotypes and diplotypes in the CYP3A cluster. The authors concluded that environmental factors explain the majority of CYP3A activity variations.

In contrast, a polymorphism of the P450 oxydoreductase (POR), an electron-donating flavoprotein that is necessary for the normal activity of all CYP450s, has been well correlated with CYP3A activity. Indeed, the POR\*28 TT genotype was associated with a 1.6-fold increase in CYP3A activity compared with POR\*28 C carriers ( $p = 0.004$ ). More studies assessing the correlation between clopidogrel antiplatelet potency, CYP3A phenotype, and/or POR\*28 SNPs are expected to specify the role of CYP3A in clopidogrel effects *in vivo* [58, 59].

#### **CYP2C9 Polymorphism**

Since CYP2C9 seems to play a role in clopidogrel bioactivation, the influence of CYP2C9 genetic variants on clopidogrel effect was also investigated. In 2007, Brandt *et al.* showed in healthy volunteers ( $n=74$ ) receiving a loading dose of 300 mg clopidogrel that the presence of loss-of-function CYP2C9 alleles was correlated with poor response and low active metabolite concentrations of clopidogrel [60]. Harmsze *et al.* showed that CYP2C9\*3 carriage was responsible for high platelet reactivity and poor response in 428 patients undergoing elective coronary stenting after maintenance or loading dose of clopidogrel [61].

### P2Y12 Polymorphism

Several polymorphisms of the gene coding for the P2Y12 protein, target of thienopyridines, have been described. A haplotype associated with ADP-induced platelet aggregation in healthy subjects drew particular attention [62]. However, studies addressing the issue of a modulation of the biological effect of clopidogrel by variants of the P2Y12 gene gave conflicting results [63], and trials addressing the issue of a clinical impact of genetic variants of the P2Y12 receptor gave negative results [39].

Altogether, it seems unlikely that genetic variants of the P2Y12 receptor have a major impact in modulating clopidogrel responsiveness. However, this view has been challenged recently [64].

### PHARMACOLOGY OF PRASUGREL

Prasugrel is a new thienopyridine prodrug with a stronger platelet inhibition effect and less variability than clopidogrel [65]. The mean maximal concentration obtained of the active metabolite of prasugrel is around 500 ng/ml within 0.5 h after 60 mg oral administration [29]. In healthy volunteers receiving 60 mg prasugrel followed by a 7-days course of 10 mg daily, the mean maximal plasma concentrations were around 80ng/ml [60]. The mechanism of action of prasugrel is identical to that of clopidogrel: prasugrel's active metabolite (R-138727) irreversibly inhibits the P2Y12 receptor and thus decreases a main amplification pathway of platelet function. However, although the active metabolite of prasugrel inhibits the P2Y12 receptor with the same potency as the active metabolite of clopidogrel [66], the greater antiplatelet effect of prasugrel is related to a more efficient generation of the active metabolite. While 85% clopidogrel is transformed by esterases into an inactive carboxylic acid, prasugrel is transformed by the carboxylesterases to form an inactive intermediate compound, which is further bioactivated by different CYPs [57] Fig. (2).

Rehmel *et al.* showed in an *in vitro* study that CYP3A is the main CYP involved in the bioactivation of prasugrel. Using recombinant CYPs, CYP3A produced the highest amount of active metabolite with a minor participation of CYP2B6, 2C9, 2C19, and 2D6. Moreover, *in vitro* addition of ketoconazole, a CYP3A specific inhibitor, reduced the formation of prasugrel active metabolite by 33–86% [67].

Brandt *et al.* assessed the impact of common polymorphism of CYP2C19 and CYP2C9 on the pharmacokinetic and pharmacody-

namic response to clopidogrel and prasugrel. They showed that the loss-of-function polymorphisms of both CYP2C19 and CYP2C9 induced a diminution of formation of the active metabolite of clopidogrel, but not prasugrel [60].

Mega *et al.* examined the association of the genetic variants of different CYPs with various cardiovascular outcomes in a cohort of 1466 patients with ACS included in the TRITON-TIMI 38 trial and treated with prasugrel. No association between different CYPs genotypes and risk of cardiovascular events like cardiovascular death, myocardial infarction, or stroke was observed. They also investigated the relation between CYPs genetic variants, plasma concentrations of active prasugrel drug metabolite, and platelet inhibition in response to prasugrel in 238 healthy subjects. No difference between carriers and noncarriers of reduced-function allele was observed in the response to prasugrel for any of the CYP genes tested [68].

### DRUG-DRUG INTERACTIONS

#### Interactions Involving Clopidogrel

Clopidogrel is metabolized principally by CYP1A2, 2C9, and 2C19 to produce the intermediate metabolite and by CYP3A4, 2B6, and 2C19 for the formation of the active metabolite [37]. In 1994, Savi *et al.* showed that the CYP1A subfamily was mainly involved in the bioactivation of clopidogrel by measuring the antiplatelet effect of clopidogrel in rats [32]. The role of CYP1A2 was further supported by a study showing an increase antiplatelet effect of clopidogrel in 104 cardiovascular patients who were current smokers compared to 155 nonsmokers [69]. Finally, the results of a subgroup of the CLARITY-TIMI 28 trial involving only smokers with myocardial infarction showed that smoking, which induces CYP1A2, was associated with improved clinical outcomes such as myocardial infarction or urgent revascularization. Those who smoked more than one-half pack/day seem to have a far greater reduction in primary endpoint (composite of TIMI, flow grade 0 or 1, death or recurrent MI) ( $p < 0.0001$ ). However, this study had a main limitation: the drugs taken by the patients that could interfere with clopidogrel response were not taken into account (statins, PPIs, etc) [70].

CYP2C19 is involved in the two-step bioactivation of clopidogrel. Consequently, drugs that inhibit or induce this CYP could potentially interfere with the clopidogrel metabolism. An interac-

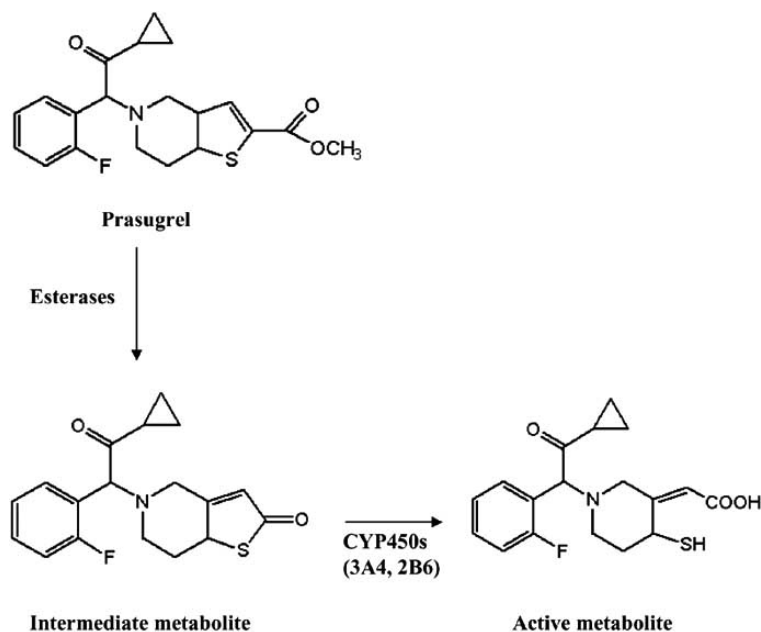


Fig. (2). Pathways of prasugrel metabolism.

tion was largely debated in recent literature between clopidogrel and PPIs. In fact, PPIs are often used with clopidogrel to prevent the increased risk of intestinal bleeding related to antiplatelet therapy.

The most used PPIs are omeprazole, esomeprazole, lansoprazole, rabeprazole, and pantoprazole. All these show a similar efficacy in the reduction of the gastric acidity and are prescribed for similar indications [71]. Omeprazole, esomeprazole, lansoprazole, and pantoprazole are mainly metabolized via CYP2C19 and partially by CYP3A4. Rabeprazole is mainly metabolized by a nonenzymatic sulfoxide reaction even if a low participation of CYP2C19 and CYP3A4 is observed [72].

Moreover, PPIs have various potencies to inhibit CYP2C19, and are therefore characterized by different inhibition constants (Table 1) [73].

Lansoprazole is the most potent inhibitor ( $k_i=0.45 \mu\text{M}$ ), while pantoprazole is the weakest one ( $k_i=69.4 \mu\text{M}$ ). Since the mean biological effect of clopidogrel is reduced in carriers of the loss-of-function CYP2C19 allele, phenotypic inhibition of CYP2C19 activity by PPIs could lead to a reduction of the clopidogrel antiplatelet effect.

Recent epidemiological case-control studies have suggested a clinically meaningful adverse drug-drug interaction between clopidogrel and PPIs. An epidemiologic case-control study in 8205 patients with ACS taking clopidogrel showed a 20.8% death or rehospitalisation for ACS incidence in patients with clopidogrel alone versus 29.8% in patients receiving clopidogrel and PPIs. The longer the patient was exposed to clopidogrel + PPIs, more were the adverse outcomes [74]. However, results might have been biased by the severity of the cardiac disease, co-morbidities, and/or co-medications. Furthermore, no distinction was made between the individual imputability of each of the different PPIs.

Juurink *et al.* have conducted another epidemiologic case-control study in 13,636 patients taking clopidogrel following ACS. A significant association between readmission for MI and current use of PPI was observed. They also showed with all PPIs, except pantoprazole, a 40% increase in the risk of recurrent MI within 90 days of hospital discharge [75]. However, in this study, the groups were not balanced in terms of cardiovascular risk factors or co-medications that may have biased the results.

Gilard *et al.* have performed an *ex vivo* study on 124 patients with coronary stent, who received aspirin and clopidogrel. The *ex vivo* impact of omeprazole was a significant increase in the platelet reactivity index (PRI) derived from the VASP (vasodilator stimulated-phosphoprotein) assay – a specific test to evaluate thienopyridines response – from 39.8% to 51.4% for patients with clopidogrel alone, and clopidogrel with omeprazole after only one week of treatment [76].

In contrast, Siller-Matula *et al.* have investigated the effects of pantoprazole and esomeprazole on clopidogrel platelet inhibition. The *ex vivo* randomized study in 300 patients taking clopidogrel and investigating their coronary permeability a year after infarction showed no significant differences in PRI and whole blood-platelet aggregation (agg) in the group with clopidogrel alone (PRI=49%;

agg=41U), clopidogrel and esomeprazole (PRI=54%; agg=42U), or clopidogrel and pantoprazole (PRI=50%; agg=47U) ( $p=0.382$ ) [77].

The only randomized trial (COGENT trial) addressing the clinical effect of the administration of clopidogrel with a PPI, allocated ACS patients to a combination of clopidogrel-omeprazole with a delayed-release formulation (CGT-2168) or to clopidogrel only. The trial was designed to assess whether PPIs reduce the incidence of gastro-intestinal bleeding while monitoring cardiovascular events as a clinical endpoint. The COGENT trial was terminated early due to financial reasons. Despite this early cessation (3627 patients included out of roughly 5000 planned), there was a significant reduction in the incidence of gastro-intestinal bleedings (relative risk [RR] = 0.55, 95% confidence interval [CI] 0.36–0.85), while there was no increase in cardiovascular events in the omeprazole group. These data seem reassuring, however the trial was underpowered to show a difference in cardiovascular events and the exposure to PPI was too short to draw any firm conclusion on the safety of a combined use of clopidogrel and omeprazole [78]. The Food and Drug Administration (FDA) has issued safety recommendations asking physicians to avoid the nonindicated prescription of PPI and to use in priority, when required, drugs that have less interaction with clopidogrel such as pantoprazole. Similar recommendations have been issued by the European Medicines Agency (EMA) for Europe, and the Medicines and Healthcare Products Regulatory Agency (MHRA) for the United Kingdom.

The clinical studies assessing the interaction between clopidogrel and PPIs are thereby contradictory, and more randomized controlled trials are needed. At the Thrombolytic Cardiovascular Therapeutics (TCT) meeting, following the COGENT presentation, Bhatt said that if a physician is concerned about the fact that the release kinetics might be different [with currently available formulations of either agent] clopidogrel could be given in the morning and PPI at night [79]. Clopidogrel and PPIs having short half-life period, a 12-h delay between the administration of clopidogrel and omeprazole could be a therapeutic alternative, even though this hypothesis must be clinically confirmed.

A recent meta-analysis addressed the issue of the clinical impact of PPI use in cardiovascular patients [80]. PPI users (42% [ $n=19614$ ]) displayed increased risk for MACE (21.8% vs. 16.7%; OR: 1.41; 95% CI: 1.34 to 1.48;  $p<0.001$ ) and mortality (12.7% vs. 7.4%; OR: 1.18; 95% CI: 1.07 to 1.30;  $p<0.001$ ;  $n=23977$ ) compared with nonusers. However, cardiovascular risk significantly influenced the impact of PPI use. Indeed, the influence of PPI was highly heterogeneous across studies and may represent the fact that patients treated with PPIs are likely to be at higher risk for ischemic events. It is thus unclear how much PPI use is a marker of more severe morbid conditions and of higher risk for adverse outcomes.

CYP3A (3A4 and 3A5) substrates or inhibitors could also interact with clopidogrel as this CYP is crucial to the second step of clopidogrel bioactivation. For example, calcium channel blockers (CCBs) are substrates and inhibitors of CYP3A. Siller-Matula *et al.* assessed, in an observational study, the responsiveness to clopidogrel in 200 patients undergoing percutaneous coronary intervention with or without CCBs treatment. They showed that the presence of CCBs allowed an impaired clopidogrel response when

**Table 1. Pharmacokinetic Parameters for the Inhibition of CYP2C19 (C<sub>max</sub> and K<sub>i</sub>) by Different PPIs**

Proton Pump Inhibitor	C <sub>max</sub> (μM)	K <sub>i</sub> (μM) to CYP2C19
Omeprazole 20mg	EM= 1.6±1.0 / PM= 3.1±0.9	6.2±0.8
Esomeprazole 40mg	EM= 5.2 / PM= 6.7	8.6±1.0
Lansoprazole 30mg	EM= 2.2±0.7 / PM= 4.8	0.45±0.07
Pantoprazole 40mg	EM= 5.4±1.4 / PM= 9.7	69.4±9.2
Rabéprazole 20mg	EM= 1.2±0.7 / PM= 1.7±0.9	21.3±2.8

measured by the VASP assay (PRI=61±18% vs. 48±21% in patients with and without CCBs, respectively (p=0.001)). The rate of cardiovascular events (death from cardiovascular causes, nonfatal myocardial infarction, stent thrombosis, and revascularization) was found 3.9-fold higher in patients with CCBs treatment than in patients without CCBs. They also realized *in vitro* experiments by addition of CCBs in plasma samples from patients taking clopidogrel and they did not identify a significant diminution of PRI when compared with the control group [81]. Therefore, CCBs did not alter the platelet function directly, but could have interfered *in vivo* with clopidogrel via CYP3A by a competitive mechanism.

Statins are substrates of CYP3A, and some studies have assessed the possibility of interaction between clopidogrel and statins. Early *in vitro* studies assessed this potential interaction. Neubauer *et al.* measured the antiplatelet effect by flow cytometry in 47 patients with coronary disease and treated with clopidogrel (300 mg loading dose followed by 75 mg daily) with or without statin treatment. A pretreatment with statin diminished the antiplatelet effect of clopidogrel at 5 h after a loading dose (43.3±19.0% vs. 61.2±18.2% in control group; p=0.01) and in the maintenance phase (48 h after a loading dose following by maintenance dose) (58.9±21.0% vs. 70.6±12.2% in control group; p=0.01) [82]. Mach *et al.* compared the effect of different statins on the antiaggregating effect of clopidogrel in 21 healthy volunteers, who received clopidogrel with each statin during one week. The *ex vivo* platelet function was measured and the results showed that simvastatin 20 mg (p=0.037) and fluvastatin 80 mg (p<0.01) interfered with the antiaggregation produced by clopidogrel, whereas atorvastatin 20 mg, pravastatin 40 mg, and rosuvastatin 10 mg did not allow any change in platelet function with clopidogrel treatment [83]. Lau *et al.* assessed the effect of atorvastatin treatment on platelet activation in 44 patients on clopidogrel with coronary artery stent implantation and showed that atorvastatin attenuated dose-dependently the antiaggregating effect produced by clopidogrel (34±23, 58±15 (P<0.027), 74±10 (P<0.002), and 89±7 (P<0.001) in the presence of clopidogrel and 0, 10, 20, and 40 mg of atorvastatin) [35]. Wenaweser *et al.* in a prospective study assessed the effect of atorvastatin and pravastatin on platelet aggregation in patients with or without (control) coronary stent thrombosis. These patients were treated by aspirin and clopidogrel and then by atorvastatin or pravastatin. They showed no impact of statins treatment on the effect of the dual antiplatelet therapy with aspirin and clopidogrel [84]. These data were confirmed by Trenk *et al.* who investigated the effect of statins on antiplatelet aggregation by clopidogrel 600 mg. They showed that simvastatin and atorvastatin neither changed the antiplatelet activity of clopidogrel nor the clinical outcomes associated with a reduced activity of clopidogrel [85]. Blagojevic *et al.* have realized a cohort study in 10,491 patients who were prescribed clopidogrel after a PCI, 43.5% of whom were receiving a statin. Cardiovascular events (death, myocardial infarction, unstable angina, repeat revascularization, or cerebrovascular events) were assessed. No association between the clopidogrel and CYP3A-metabolized statins group and an increase of adverse cardiovascular events was demonstrated [86]. These data suggest that statins do not influence clinical outcomes of such cardiovascular events in patients treated with clopidogrel, even though some early studies showed a decreased biological effect of clopidogrel in presence of statins treatment.

The reasons for these discrepancies are not clear and may be related to the multifactorial causes of the variability in the individual response to clopidogrel. As mentioned before, factors such as age, BMI or lipid levels may impact on the variability of clopidogrel responsiveness, together with several genetic variants of proteins involved in absorption or metabolism of the drug.

Ketoconazole, a potent CYP3A inhibitor, was used to assess the effect of CYP3A inhibition on clopidogrel pharmacodynamics in 18 healthy volunteers receiving 400mg ketoconazole or not (control)

with 300mg clopidogrel LD on day 1 and 75mg daily MD during 6 days. Ketoconazole significantly reduced the clopidogrel active metabolite exposure and the antiplatelet activity of clopidogrel. Therefore, clopidogrel active metabolite formation appears to strongly depend on CYP3A activity [87].

Clopidogrel and its 2-oxo metabolite also inhibited CYP2B6 *in vitro* (Ki= 0.72±0.33 and 1.13±0.20, respectively) [88] whereas clopidogrel inhibited in a lesser extent CYP2C9 in another *in vitro* study [89]. However more clinical studies are needed to confirm an eventual drug–drug interaction between clopidogrel and CYP2B6 or CYP2C9 substrates.

### Interactions Involving Prasugrel

Hagihara *et al.* compared the inhibitory potentials of some CYPs by ticlopidine, clopidogrel, and prasugrel. They showed that ticlopidine inhibited all the CYPs tested (CYP2B6, 2D6, and 2C19), whereas clopidogrel only inhibited CYP2B6 and 2C19 and prasugrel did not inhibit any of those CYPs. Similarly, the active metabolites of clopidogrel and prasugrel had no inhibitory propensity. They concluded that prasugrel had a lower potential for drug–drug interaction than clopidogrel or ticlopidine [90].

This finding confirmed the results of Rehmel *et al.* in an *in vitro* study in which they showed that the two major metabolites of prasugrel did not inhibit the CYP1A2, 2C9, 2C19, 2D6, and 3A [67].

O'Donoghue *et al.* have investigated the possible interaction between prasugrel and PPIs in comparison with clopidogrel. They assessed the effect of the PPI prescription on the platelet function and clinical outcomes in patients with elective PCI. The inhibition of platelet aggregation was reduced by the concomitant use of clopidogrel and PPIs (p=0.02), whereas a modest difference (p=0.054) was observed for prasugrel. Moreover, no association was found between PPI concomitant prescription and cardiovascular death, stroke, or myocardial infarction and clopidogrel or with prasugrel [91].

The second step of prasugrel metabolism involves mainly CYP3A4 and CYP2B6. Therefore, relevant drug–drug interactions would have to be with drugs metabolized by these CYPs through a competitive mechanism, or with drugs that induce or inhibit these CYPs.

In a crossover randomized study, Farid *et al.* assessed the platelet aggregation in healthy volunteers receiving a loading dose (60 mg) followed by a maintenance dose (15 mg) of prasugrel with or without 400 mg ketoconazole (a potent inhibitor of CYP3A). Ketoconazole reduced the maximum plasma concentration of prasugrel active metabolite but neither had an impact on the exposure to prasugrel active metabolite nor on the antiplatelet effect of prasugrel [86]. This discrepancy may be related to the involvement of other minor metabolic enzymatic pathways (CYP2B6, CYP2C9 and CYP2C19) in prasugrel metabolism that may produce prasugrel active metabolite from prasugrel intermediate metabolite in the liver when CYP3A activity is reduced.

The effect of a CYP3A inducer, rifampicin (600 mg), was tested in healthy male volunteers taking prasugrel (LD=60 mg followed by 10 mg/day) by measuring the platelet inhibition and determining the active metabolite concentration. Rifampicin did not influence the production of prasugrel active metabolite, but reduced the inhibition of platelet aggregation after a maintenance dose (p>0.001 between prasugrel+rifampicin group and control group, 24 h after the 6<sup>th</sup> day of prasugrel treatment). This inhibition could however be due to an artefact with the light transmission aggregometry method due to the coloration of rifampicin [92]. An interaction could therefore potentially occur with CYP3A inducers. However, more randomized controlled studies are needed to confirm these preliminary results.

Antiretrovirals are known to be strong CYP3A4 and 2B6 inhibitors and thus could interfere with prasugrel bioactivation by

inhibition of its metabolism mediated by these CYPs, and further studies should be realized to test this hypothesis.

## CONCLUSIONS

Clopidogrel is a cornerstone in the prevention of ischemic events in patients with ACS and those undergoing PCI. However, there is interindividual variability of its biological potency in treated patients. This variability is partly explained by several factors, including genetic polymorphisms that have been recently studied; some variant alleles of CYPs were shown to be associated with the biological effect of clopidogrel and the CYP2C19\*2 variant was associated with worse clinical outcomes in patients treated with clopidogrel. The P-gp transporter also seems to play a role in this variability. Even though these two components could explain part of the variability of the response to clopidogrel, other environmental and interindividual factors such as noncompliance, comorbidities, high BMI, increased platelet turnover, and up-regulation of metabolic pathways could play a role in this variability.

In waiting for randomized controlled studies investigating drug–drug interactions involving the different CYPs playing a role in clopidogrel metabolism, it seems reasonable to avoid potent CYPs inhibitors or inducers such as PPIs that inhibit CYP2C19, especially in high risk populations, and to prefer H2 antagonists. The beneficial effect of a delay between clopidogrel and PPI intake remains to be established.

Prasugrel shows a better efficacy than clopidogrel to prevent cardiovascular events and seems to be less subjected to drug–drug interaction in comparison with clopidogrel, as shown with PPIs. Nonetheless, it also increases the risk of bleeding that needs to be taken into account before prescribing it. Therefore, the FDA recommends avoiding prasugrel prescription in some subgroups of patients: elderly, those with body weight < 60 Kg and those with a history of stroke or transient ischemic attack.

Tailored maintenance treatment based on each patient's individual biological response and/or genetic testing may be beneficial for the long-term prognosis to minimize ischemic and hemorrhagic events. The most relevant tests and cut-off values to define poor responsiveness to clopidogrel have to be further investigated, and special attention is required to avoid drug–drug interactions.

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