P2Y₁₂ Receptor Inhibitors in Acute Coronary Syndromes: From the Research Laboratory to the Clinic and Vice Versa

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Key Words

P2Y₁₂ receptor inhibitor · Acute coronary syndrome · Platelet activation

Abstract

The P2Y₁₂ receptor plays a pivotal role in platelet activation and aggregation through a complex cascade of actions. Laboratory and clinical data have convincingly shown the benefit of P2Y₁₂ inhibition combined with aspirin in patients with acute coronary syndrome (ACS)/undergoing percutaneous coronary intervention (PCI). Newer agents – like prasugrel, ticagrelor, and cangrelor – provide more consistent, faster, and stronger platelet inhibition than clopidogrel. In large clinical trials newer agents have resulted in fewer ischemic complications (though with increased bleeding potential) than clopidogrel. High-risk subpopulations like ST-segment elevation myocardial infarction, diabetes, chronic kidney disease, elderly, and low body weight patients have been identified. A ‘return to the laboratory’ has been observed recently, with several pharmacodynamic studies being performed particularly in these cohorts. This interplay between research laboratory and clinical data may lead to a more efficient and safer use of P2Y₁₂ inhibitors.

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Introduction

Several lines of evidence from basic research and clinical studies underline the central role of platelet activation in the pathogenesis of acute coronary syndromes (ACS). Percutaneous coronary intervention (PCI), the main interventional treatment in ACS patients, constitutes a powerful thrombogenic stimulus in itself. The P2Y₁₂ receptor is a Gi-coupled adenosine diphosphate (ADP) receptor, which plays a pivotal role in platelet activation and aggregation through a complex cascade of actions. It is therefore obvious why the P2Y₁₂ receptor has become a molecular target in the development of antiplatelet drugs administered to ACS/PCI patients. Laboratory and clinical data have convincingly shown the benefit of P2Y₁₂ inhibition combined with aspirin. In the last 2 decades several P2Y₁₂ inhibitors have been introduced in clinical practice, with thienopyridine clopidogrel being the most widely used agent around the world. However, the realization of the caveats of clopidogrel, i.e. interpatient variability and a relatively weak and slow-onset antiplatelet action, has led to the development of newer, more potent agents – like prasugrel, ticagrelor, and cangrelor – which are able to provide more consistent, faster, and stronger platelet inhibition than clopidogrel and overcome the, rather commonly, observed high on-clopidogrel platelet reactivity (HPR). In the clinical field, in large

phase III trials newer agents have resulted in fewer ischemic complications (though with increased bleeding potential) than clopidogrel. Intense research efforts have focused on linking HPR with clinical outcomes and, most importantly, on using platelet function testing (PFT) as a guide to tailored antiplatelet treatment. High-risk subpopulations like ST elevation myocardial infarction (STEMI), diabetes, chronic kidney disease (CKD), elderly, and low body weight (LBW) patients have been identified. A 'return to the laboratory' with several pharmacodynamic studies particularly in these cohorts has been observed recently in an effort to refine the applied antiplatelet treatment.

In this review we focus on the interplay between research laboratory and clinical data on P2Y₁₂ inhibitors in ACS/PCI patients. Recent pharmacodynamic studies may be paving the way for a more efficient and safer use of P2Y₁₂ inhibitors.

Platelets: Their Central Role in ACS/PCI

Platelet-rich thrombus formation induced by plaque rupture and endothelial cell erosion is the underlying pathogenetic mechanism in most cases of ACS. Particularly in STEMI patients, platelets are highly activated and represent a main constituent of 'fresh' thrombi [1]. Activated platelets release thromboxane A₂ and ADP, which is stored in and secreted from dense granules in platelets, amplifying also the response to other agonists. The P2Y₁₂ receptor plays a central role in platelet activation amplification and the ADP-P2Y₁₂ interaction is crucial for stable, platelet-rich thrombus generation at the site of the vessel wall injury [2]. In an in vivo mesenteric artery injury model, the time for the appearance of the first thrombus was prolonged and only small, unstable thrombi formed in P2Y₁₂^{-/-} mice without reaching an occlusive size. Platelet adhesion to von Willebrand factor was impaired and P2Y₁₂^{-/-} platelet activation in response to ADP or a PAR-4-activating peptide was reduced, supporting the notion that P2Y₁₂ is involved in several key steps of thrombosis, like platelet adhesion/activation, thrombus growth, and stability [3].

During PCI, the commonest form of interventional treatment for coronary artery disease, the subendothelial matrix is exposed and platelet adhesion is promoted. Platelets are activated by collagen and von Willebrand factor binding to specific membrane receptors, while tissue factor exposure leads to thrombin generation. Thromboxane A₂ and ADP are released from activated platelets. Therefore, PCI per se constitutes a powerful thrombogenic stimulus [4].

P2Y₁₂ Receptor Inhibitors

Platelet inhibition via P2Y₁₂ ADP receptor blocking is a key target in the prevention of ischemic complications in patients with ACS/PCI with stents. Attenuation of platelet aggregation induced by several agonists, modulation of platelet procoagulant activity, thrombin generation, P-selectin expression, soluble CD40L, and inflammation marker release are some of the consequences of effective P2Y₁₂ blockage [2, 5]. Several drugs targeting the P2Y₁₂ receptor have been developed, like the thienopyridines ticlopidine, clopidogrel, and prasugrel that selectively and irreversibly inhibit the P2Y₁₂ receptor. Moreover, ticagrelor is a cyclo-pentyltriazolo-pyrimidine that inhibits the P2Y₁₂ receptor reversibly, while cangrelor, an ATP analogue, is a reversible, parenteral P2Y₁₂ inhibitor. The development of elinogrel – an agent for both oral and parenteral use – has been terminated.

P2Y₁₂ Inhibition Combined with Aspirin: Laboratory and Clinical Data

In ex vivo experimental models of arterial thrombosis using human blood samples, ticlopidine or clopidogrel combined with aspirin significantly enhanced the anti-thrombotic effect of each drug alone [6, 7]. In the clinical scenario of ACS, in 12,562 patients participating in the Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) trial, the combination of clopidogrel and aspirin therapy reduced the incidence of the composite end point of death, myocardial infarction (MI), and stroke compared to aspirin monotherapy (relative risk with clopidogrel compared to placebo 0.80; 95% CI 0.72–0.90, *p* < 0.001). However, significantly more patients receiving clopidogrel had major bleeding (relative risk 1.38, *p* = 0.001) [8].

Clopidogrel Response Variability

Clopidogrel, the most widely used ADP receptor inhibitor, undergoes a two-step metabolic transformation regulated by the CYP450 system before binding to the P2Y₁₂ receptor. Gurbel et al. [9] first reported clopidogrel response variability using conventional platelet aggregometry and flow cytometry studies in patients undergoing PCI who had received a 300-mg loading dose (LD) followed by a 75-mg daily maintenance dose (MD) of clopidogrel. The prevalence of resistance varied with the time from clopidogrel loading. Variable and insufficient

active metabolite generation seems to be the main explanation for clopidogrel response variability and HPR, respectively. Several additional factors, like ethnicity, age, diabetes mellitus, renal dysfunction, obesity, acuity of the disease, drug-drug interactions, and smoking, have been identified to affect an individual's response to clopidogrel [10]. The presence of genetic polymorphisms also determines the extent to which clopidogrel inhibits ADP-induced platelet activation. The *CYP2C19**2 loss-of-function allele is the most commonly identified polymorphism resulting in reduced clopidogrel active metabolite production and subsequent antiplatelet activity, although a limited (12%) amount of the observed variation is attributed to it [11]. More disconcerting is the fact that, even after controlling for known factors affecting the pharmacokinetics/pharmacodynamics of clopidogrel, most of their variation remains unexplained [12].

Overcoming HPR: P2Y₁₂ Inhibitors beyond Clopidogrel – Pharmacodynamic Studies

Increasing the usual 75 mg/day clopidogrel dose to 150 mg/day seems to be a reasonable approach to overcome HPR. However, a suboptimal clopidogrel response may still be observed in a variable proportion of patients [13], pointing to the need for more potent agents to achieve adequate platelet inhibition. Prasugrel, by undergoing a single metabolic step in the liver necessary for its activation, provides faster, stronger, and more consistent platelet inhibition than clopidogrel [14]. In patients with HPR, prasugrel was found to be more effective than a double dose of clopidogrel early or long after PCI [15, 16]. This advantage of prasugrel was more prominent in *CYP2C19**2 carriers [15]. Ticagrelor, also a direct acting agent, was able to overcome HPR in all patients who were nonresponders to clopidogrel [17]. Of note, variability in the response to prasugrel has also been observed – though to a lesser extent than with clopidogrel – with an HPR-on-prasugrel prevalence of 5–25% depending on, among other factors, the method of PFT and the timing after loading [18, 19]. No case of HPR while on chronic ticagrelor has been reported so far, and platelet reactivity variation with this agent is more limited [20].

Large Clinical Studies

Following the results of the CURE trial, dual antiplatelet treatment with clopidogrel combined with aspirin became the standard of care in patients with PCI

and/or ACS [8]. However, a considerable number of patients continue to experience adverse outcomes, including both bleeding and recurrent ischemic events. A plausible explanation for the latter is that the efficacy of clopidogrel is hampered by a slow and variable transformation of the prodrug to the active metabolite and the modest and variable platelet inhibition mentioned above. Pharmacodynamic data provided the basis for large clinical trials of regimens with more potent antiplatelet activity in order to overcome caveats in clopidogrel use while simultaneously assessing their bleeding potential. In the Clopidogrel Optimal Loading Dose Usage to Reduce Recurrent Events/Optimal Antiplatelet Strategy for InterventionS (CURRENT-OASIS) 7 trial, double clopidogrel (600-mg LD/150-mg MD for 7 days followed by a 75-mg MD) was compared to the standard (300-mg LD/75-mg MD) dose regimen. Although in the overall population no difference was found between the 2 clopidogrel regimens, in the PCI cohort (n = 17,263) the double clopidogrel dose was accompanied by an improvement in the primary outcome of cardiovascular death, MI, or stroke at 30 days and definite stent thrombosis with an adjusted hazard ratio (HR) of 0.86 (95% CI 0.74–0.99, p = 0.039) [21]. Major bleeding was increased with the double clopidogrel dose (HR 1.41; 95% CI 1.09–1.83, p = 0.009). In the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel (TRITON)-TIMI 38 trial in 13,608 patients with moderate-to-high-risk ACS with scheduled PCI, prasugrel (60-mg LD/10-mg MD) compared to clopidogrel (300-mg LD/75-mg MD) reduced the primary efficacy end point (death from cardiovascular causes, nonfatal MI, or nonfatal stroke) at 15 months (HR 0.81; 95% CI 0.73–0.90, p < 0.001). Major bleeding was increased (HR 1.32; 95% CI 1.03–1.68, p = 0.03) [22]. In the Study of Platelet Inhibition and Patient Outcomes (PLATO) trial, ticagrelor (180-mg LD/90-mg MD b.i.d.) compared to clopidogrel (300- to 600-mg LD/75-mg MD) in 18,624 patients reduced the primary end point (a composite of death from vascular causes, MI, or stroke) at 12 months (HR 0.84; 95% CI 0.77–0.92, p < 0.001). The rate of death from any cause was reduced with ticagrelor (4.5 vs. 5.9% with clopidogrel; p < 0.001). Ticagrelor also was associated with a higher rate of major bleeding not related to coronary artery bypass grafting (4.5 vs. 3.8%, p = 0.03) [23]. The results of these pivotal trials offer the basis for the more potent antiplatelet regimen recommendations in patients with ACS/PCI according to clinical guidelines.

Linking Clinical Outcomes to HPR: Rationale for Tailoring Treatment

Accumulating data from several studies underscores the importance of HPR as a factor predicting ischemic risk. In the largest study so far, i.e. the Assessment of Dual Anti-Platelet Therapy with Drug-Eluting Stents (ADAPT-DES) registry of 8,665 patients (>50% with ACS), an HPR defined as >208 P2Y₁₂ reaction units (PRU) by the VerifyNow (Accumetrics Inc., San Diego, Calif., USA) P2Y₁₂ function assay was linked beyond any doubt to stent thrombosis (adjusted HR 2.49; 95% CI 1.43–4.3, $p = 0.001$) and MI (adjusted HR 1.42; 95% CI 1.09–1.86, $p = 0.01$), and it was inversely related to bleeding (adjusted HR 0.73; 95% CI 0.61–0.89, $p = 0.002$). However, the impact of HPR on mortality was neutral, emphasizing the counterbalancing effects of hemorrhagic and ischemic complications [24]. Thresholds of HPR after PCI, predicting the ischemic risk, have been defined by ROC analyses using the following criteria: (1) PRI >50% by vasodilator-stimulated phosphoprotein; (2) >235–240 PRU by the VerifyNow P2Y₁₂ assay; (3) >46% maximal 5 $\mu\text{mol/l}$ ADP-induced aggregation, and (4) >468 arbitrary aggregation units/min in response to ADP by a Multiplate analyzer [25]. More important than the identification of a heightened risk, however, is the improvement in outcomes achieved by modifying this risk, which has not been accomplished so far. Prospective randomized trials of personalized antiplatelet therapy have failed to improve clinical outcomes [26–28]. Low-risk populations and low event rates have been implicated.

At the other end of the spectrum, low platelet reactivity while on thienopyridine has been linked to an increased bleeding risk. Therefore, a therapeutic window or ‘sweet spot’ concept has been proposed for P2Y₁₂ inhibitor therapy. In a recent study in 1,542 patients with PCI/ACS treated with thienopyridines, a vasodilator-stimulated phosphoprotein index $\leq 10\%$ predicted bleeding events during a 6-month follow-up with a sensitivity of 17% and a specificity of 97% [29].

Special Populations: Pharmacodynamic and Clinical Data

STEMI

In patients suffering a STEMI, platelets are greatly activated and effective platelet inhibition constitutes the cornerstone of adjunctive-to-primary PCI pharmacologic treatment. In the CURRENT-OASIS 7 trial, in the

STEMI subgroup ($n = 6,365$) – mostly treated with primary PCI – the reduction of the primary end point with the high clopidogrel dose was nominally nonsignificant, i.e. 4.2 versus 5% (HR 0.83; 95% CI 0.66–1.05, $p = 0.117$). However, the results were consistent with the overall trial and the clinical presentation (STEMI vs. non-STEMI) did not affect them (p for interaction = 0.817) [21]. In the (TRITON)-TIMI 38 trial, in the STEMI cohort ($n = 3,534$), prasugrel compared with clopidogrel resulted in a reduction of the primary end point at 30 days (HR 0.68; 95% CI 0.54–0.87, $p = 0.0017$) and at 15 months (HR 0.79; 95% CI 0.65–0.97, $p = 0.0221$) [30]. This finding was consistent with the overall TRITON TIMI-38 results, with no interaction noted between the presenting syndrome and the benefits of prasugrel. TIMI major bleeding unrelated to coronary artery bypass grafting did not differ between groups at 30 days or 15 months. However, most of the benefit seen with prasugrel in the whole STEMI group was driven by its anti-ischemic superiority over clopidogrel in the secondary and not the primary PCI group. In the PLATO trial, in the STEMI cohort of patients with ST elevation or left bundle branch block, the primary end point occurred in 9.4 and 10.8% of patients receiving ticagrelor and clopidogrel, respectively (HR 0.87; 95% CI 0.75–1.01, $p = 0.07$) [31]. There was no interaction between presentation with ST elevation or left bundle branch block and randomized treatment, and these results were consistent with the overall PLATO findings.

Going back to the laboratory, a detailed analysis of the onset of action and the magnitude of platelet inhibition after a 600-mg LD of clopidogrel in primary PCI patients was first described by Heestermans et al. [32]. The absolute plasma concentrations of unchanged clopidogrel (C_{max} , AUC) were significantly lower in STEMI patients compared to healthy volunteers and a variably impaired intestinal absorption was implicated. Four hours after the LD, only a minor decrease (6–7%) in platelet aggregation was seen. In a study by our group, in primary PCI patients, platelet reactivity assessment with the VerifyNow assay 2 h after a 600-mg LD of clopidogrel revealed that 64.5% of patients exhibited HPR (defined as ≥ 235 PRU) [33].

The pharmacokinetic and pharmacodynamic advantages of prasugrel or ticagrelor over clopidogrel are of particular interest when pretreatment is not possible and fast inhibition of platelet aggregation is desirable, as is the case for primary PCI. Clinical data in STEMI cohorts preceded the pharmacodynamic ones showing better anti-ischemic protection provided by a potent regimen versus clopidogrel without an increase in major bleeding. In the

Fig. 1. Individual values of platelet reactivity at 0, 1, 2, 6, and 24 h and day 5 postrandomization, as assessed with the Verify-Now assay (in PRU). The lines represent medians and the error bars represent quartiles 1–3. High on-treatment platelet reactivity thresholds are shown. Reprinted with permission from Alexopoulos et al. [35].

field of pharmacodynamics, Valgimigli et al. [34] first reported a significantly lower inhibitory platelet activity to 20 $\mu\text{mol/l}$ ADP stimulation at 30 min postprasugrel in STEMI patients compared to tirofiban-treated patients. A suboptimal degree of platelet inhibition for at least 2 h following prasugrel was found. In STEMI patients loaded with 600 mg clopidogrel and 2 h later exhibiting HPR, reloading with 60 mg prasugrel resulted in 40% of patients retaining HPR 2 h after reloading, at a time point when one would expect adequate platelet inhibition based on non-STEMI patients' data [33]. In the first direct, randomized, pharmacodynamic comparison of ticagrelor with prasugrel in primary PCI patients at 2 h post-LD, the HPR rates (cutoff 208 PRU) were 46.2 and 34.6% for ticagrelor and prasugrel, respectively [35]. Both ticagrelor and prasugrel exhibited an initial delay in the onset of their antiplatelet action (fig. 1). In an effort to bridge this initial gap, a double (360 mg) ticagrelor LD versus the standard (180 mg) LD was tested in primary PCI patients without success; no significant difference in platelet inhibition between the two regimens was found [36].

Diabetic Patients

Pharmacodynamic studies performed in diabetic patients receiving clopidogrel consistently demonstrated heightened platelet reactivity compared to nondiabetic patients, as well as an increased prevalence of HPR. Prasugrel provided a higher inhibitory platelet activity when compared even with a double dose of clopidogrel in patients with diabetes and coronary artery disease [37]. In the clinical field, a prespecified, subgroup analysis of TRITON-TIMI 38 among diabetic patients showed that prasugrel significantly reduced the incidence of the composite end point of cardiovascular death, MI, and stroke compared to clopidogrel (12.2 and 17.0%, respectively, HR 0.70; $p < 0.001$) [38]. In the subgroup analysis of the PLATO trial in diabetic patients, the reduction in the primary composite end point (HR 0.88; 95% CI 0.76–1.03), all-cause mortality, and stent thrombosis with no increase in major bleeding by ticagrelor was consistent with the overall trial results [39]. It has been proposed that prasugrel may be the preferred treatment option when diabetes is present. How-

Fig. 2. Individual platelet reactivity values according to treatment. Combined data for the pre- and postcrossover periods are depicted. Lines represent medians and error bars represent interquartile ranges. Reprinted with permission from Alexopoulos et al. [40]. Copyright of the American Diabetes Association.

Fig. 3. Platelet reactivity by treatment sequence. Data for the pre- and postcrossover periods are depicted. Error bars represent 95% CIs. Comparisons between treatments groups (solid lines) and within therapies (dotted lines) are shown (analysis of covariance models with baseline platelet reactivity as a covariate). Reprinted with permission from Alexopoulos et al. [46].

ever, there are no direct clinical outcome comparisons of ticagrelor versus prasugrel. Going back to the laboratory, we have demonstrated in a direct, pharmacodynamic comparison of ticagrelor versus prasugrel in diabetic patients exclusively a significantly lower platelet reactivity after ticagrelor compared with prasugrel (fig. 2) [40].

Chronic Kidney Disease

Patients with CKD are at an increased cardiovascular risk and cardiovascular complications represent the commonest mode of death in such patients. Pharmacodynamic studies in CKD patients have revealed an impaired response to clopidogrel. The HPR prevalence is very high, escalating with increasing stages of disease severity and predictive of adverse events after PCI [41, 42]. In patients on hemodialysis, prasugrel or ticagrelor may overcome HPR, although no outcome data on efficacy and particularly safety are available in this high-risk cohort of patients [43, 44]. In the clinical field, in patients with a creatinine clearance <60 ml/min – excluding hemodialysis – data from TRITON TIMI-38 showed a reduction in the primary end point of cardiovascular death, MI, an stroke by a nonsignificant 14% with prasugrel compared to clopidogrel [22]. In the PLATO trial, in patients with a creatinine clearance <60 ml/min – excluding hemodialysis – ticagrelor compared to clopidogrel significantly reduced the primary end point of cardiovascular death, MI, and stroke (HR 0.77; 95% CI 0.65–0.90) without an increase in major bleeding [45].

Elderly/LBW Patients

Post hoc analysis of the TRITON TIMI-38 trial identified elderly (≥ 75 years old) and LBW (<60 kg) patients as 2 subgroups where prasugrel had a ‘neutral’ effect over clopidogrel [22]. The increased anti-ischemic benefit of prasugrel over clopidogrel seems to be counterbalanced by more frequent bleeding events in these subpopulations. Returning to the laboratory, evaluation of a low (5 mg) prasugrel dose in elderly patients with ACS undergoing PCI and exhibiting HPR following standard clopidogrel treatment showed that this regimen was significantly more efficacious than 150 mg/day clopidogrel in reducing platelet reactivity and the HPR rate (fig. 3) [46]. Therefore, the use of a reduced (5 mg/day) dose of prasugrel appears to be an option for treating elderly patients, although more data on the efficacy and safety of this reg-

imen are needed. In the clinical field, the other oral, potent P2Y₁₂ inhibitor, i.e. ticagrelor, when compared to clopidogrel in the context of the PLATO trial appears to provide superior anti-ischemic action in the elderly subgroup (which is consistent with the overall trial results), with no excess in bleeding [23].

Regarding LBW patients, the extent of the reduction of platelet reactivity was similar with 5 mg prasugrel and 10 mg prasugrel in high body weight patients and prasugrel resulted in greater platelet inhibition, lower HPR, and similar bleeding rates compared to clopidogrel [47].

Parenteral P2Y₁₂ Inhibitors

Cangrelor (formerly AR-C69931MX) is a parenteral, direct acting, reversible P2Y₁₂ inhibitor. In a canine model of arterial thrombosis, cangrelor was shown to antagonize the ex vivo and in vivo aggregative actions of ADP, displaying a rapid onset and offset of action and preventing occlusive arterial thrombus formation [48]. The use of cangrelor in patients undergoing PCI has been studied in 3 phase 3 trials, i.e. Cangrelor versus Standard Therapy to Achieve Optimal Management of Platelet Inhibition (CHAMPION) PCI, CHAMPION PLATFORM, and CHAMPION PHOENIX. In a recent pooled analysis of patient level data from these 3 trials, cangrelor compared to the control (clopidogrel or placebo) reduced the primary efficacy composite end point of death, MI, ischemia-driven revascularization, or stent thrombosis at 48 h (OR 0.81;

95% CI 0.71–0.91), and stent thrombosis (OR 0.59; 95% CI 0.43–0.80). The price was an increase in GUSTO mild bleeding (16.8 vs. 13.0%, $p < 0.0001$) [49]. The rapid onset and offset of action of cangrelor makes this agent ideal for ‘bridging’ therapy in patients discontinuing oral agents in order to undergo surgery [50] and, possibly, when a rapid onset of action is needed, like in STEMI patients [35].

Future Perspectives

Because of the central role of P2Y₁₂ receptor activation in ACS/PCI patients, strong platelet inhibition will remain pivotal. For the time being, PFT does not have a defined role in the clinical routine following antiplatelet treatment. Nevertheless, PFT will more likely find a role beyond the research laboratory in high-risk subpopulations like STEMI, diabetes, CKD, elderly, and LBW patients. Further areas of interest like genotyping may still develop if the universal use of novel agents due to costs, safety, or other concerns is abandoned. Bridging strategies with fast and short acting agents like cangrelor needs further testing in clinical studies in STEMI or surgery candidate patients.

In conclusion, an interplay between research laboratory and clinical data with P2Y₁₂ inhibitors in ACS/PCI patients has been observed in recent years. Recently performed pharmacodynamic investigations may be paving the way for further clinical studies in order to achieve a more efficient and safer use of P2Y₁₂ inhibitors.

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