

Antagonizing P2Y₁₂ Receptor Inhibitors: Current and Future Options

Dietmar Trenk¹ Laura Hille¹ Stefan Leggewie¹ Christian Stratz¹ Thomas G. Nührenberg¹
Daniel Aradi^{2,3} Karsten Schrör⁴ Dirk Sibbing^{5,6}

¹Department of Cardiology and Angiology II, Clinical Pharmacology, University Heart Centre Freiburg, Bad Krozingen, Germany

²Heart Center Balatonfured, Balatonfured, Hungary

³Heart and Vascular Center, Semmelweis University, Budapest, Hungary

⁴Institute for Pharmacology and Clinical Pharmacology, University Hospital Düsseldorf, Düsseldorf, Germany

⁵Department of Cardiology, LMU München, Munich, Germany

⁶DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany

Address for correspondence Dietmar Trenk, PhD, Department of Cardiology and Angiology II – Clinical Pharmacology, University Heart Centre Freiburg - Bad Krozingen, Südring 15, D-79189 Bad Krozingen, Germany (e-mail: Dietmar.Trenk@universitaets-herzzentrum.de).

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Abstract

There are clinical scenarios where the balance between the risk of ischemic and bleeding events leads to the clinical decision to reverse the antiplatelet effect of P2Y₁₂ receptor inhibitors. These scenarios comprise emergency situations such as active severe bleeding, urgent procedures with presumed high bleeding risk, or major trauma with (anticipated) bleeding. Supplementation of platelets has been investigated in ex vivo as well as in in vivo studies. These studies indicate that the inhibition of adenosine diphosphate-induced aggregation by the irreversibly binding thienopyridine derivatives clopidogrel and prasugrel can be reversed by administration of platelet concentrates. Supplementation of platelets in patients on prasugrel is more effective if this can be transfused > 6 hours after last dosing. Studies on the reversal effect obtained by administration of platelet concentrates in patients on ticagrelor show conflicting results. Experimental data suggest that administration of serum albumin might increase the reversal effect. A monoclonal antibody fragment (PB2452) for neutralizing ticagrelor is currently in clinical development. A recently published first in man study shows that reversal of platelet inhibition occurs within 5 minutes after start of administration and the effect is maintained for 20 to 24 hours after a 16-hour infusion which is by far the most effective approach for reversal of ticagrelor.

Keywords

- clopidogrel
- prasugrel
- ticagrelor
- reversal of P2Y₁₂ receptor inhibition

Introduction

Platelet activation and aggregation play an important role in the development of ischemic events during and after acute coronary syndromes (ACSs) and percutaneous coronary interventions (PCIs).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 intense anti-

coagulant regimens stimulated clinical studies investigating novel antithrombotic regimens using two antiplatelet drugs, namely aspirin and a thienopyridine. Compared with aspirin plus anticoagulation (heparin followed by vitamin K antagonists), dual antiplatelet therapy (DAPT) with aspirin and ticlopidine which was the only available thienopyridine at that time decreased the incidence of cardiac and vascular complications after the placement of coronary artery stents.

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Moreover, the incidence of bleeding complications during follow-up was markedly decreased.⁵ DAPT with aspirin and a P2Y₁₂ receptor inhibitor became therefore the standard of care for prevention of ischemic complications in ACS patients and in patients undergoing PCI.

Ticlopidine was replaced early by the second generation thienopyridine clopidogrel due to safety concerns regarding allergy, skin, or gastrointestinal disorders.^{6,7} Clopidogrel has a similar degree of P2Y₁₂ inhibition and bleeding risk like ticlopidine but an improved safety profile. Clinical studies have demonstrated that DAPT with aspirin and clopidogrel results in a substantial rate of patients with attenuated response to clopidogrel impacting on clinical outcome.^{8–12} Large-scale clinical outcome studies combining aspirin with the P2Y₁₂ receptor antagonists prasugrel or ticagrelor in patients presenting with an ACS demonstrated a prognostic benefit by a more consistent and more potent inhibition of the P2Y₁₂ receptor compared with DAPT with aspirin plus clopidogrel.^{13,14} The reduction in ischemic events was, however, achieved at the cost of an increased rate of major bleeding events due to the exaggerated suppression of platelet reactivity.

Current European and U.S. guidelines endorse the use of DAPT after PCI with stent placement or previous myocardial infarction.^{15,16} DAPT with aspirin and clopidogrel is prescribed for patients with stable coronary artery disease (SCAD); while DAPT combining aspirin with prasugrel or ticagrelor is favored over clopidogrel for patient presenting with an ACS.

The duration of DAPT in CAD depends on the clinical presentation of the patient (stable CAD vs. ACS), a clinical assessment of the postprocedural ischemic risk, and the potential bleeding risk. Use of risk scores to guide DAPT duration has been endorsed in the recently published focused update on DAPT in CAD of the European Society of Cardiology (ESC).¹⁷

Any decision of premature interruption of DAPT must be based on sound clinical judgment and a clear understanding of the potential risk and benefits, including acute thrombotic events. Therefore, current guidelines recommend to postpone elective surgical interventions beyond the early phase of coronary intervention and stenting, that is usually a minimum of 30 days after the procedure.¹⁶ This timeframe is based upon analysis of Danish population base registries assessing the risk associated with surgery among drug-eluting stent-PCI-treated patients compared with patients with ischemic heart disease undergoing a similar surgical procedure.¹⁸ When stratified for time from PCI to surgery, surgery within the first month after PCI was associated with increased risk of ischemic events. Thus, elective surgery should be postponed to > 30 days after PCI and controlled cessation of antiplatelet therapy before surgery according to the estimated time for recovery of platelet function, which is 3 days for the reversible inhibitor ticagrelor and 5 and 7 days for the irreversible inhibitors clopidogrel and prasugrel, should be performed.

However, there are clinical scenarios where strategies for rapid establishment of hemostasis by reversal of the anti-

platelet effect are required. This comprises various emergency situations such as:

- Active bleeding, particularly severe or life-threatening bleeding into critical organs like intracranial bleeding or intraocular bleeding.
- Unscheduled, urgent, or emergent procedures that carry a presumed high bleeding risk, or procedures where the consequences of even minor bleeding would be unacceptable (e.g., spinal surgery or other neurosurgical procedures).
- Major trauma with bleeding.

No guidance based upon randomized clinical trials is available for patients on DAPT who either develop active bleeding complications or who are scheduled for urgent procedures with presumed high bleeding risk. Current ESC guidelines recommend to balance in this setting ischemic risk (e.g., indication for DAPT, time from last PCI with stenting) versus recurrent/prolonged bleeding risks.¹⁷ In severe bleeding, de-escalation of antiplatelet therapy from DAPT to single antiplatelet therapy should be considered, and if bleeding persists, stopping all antithrombotic medications is recommended. In life-threatening bleeding, all antithrombotic medications are discontinued immediately and red blood cell or platelet transfusion are mentioned as general recommendation.¹⁷

After the decision for reversal of platelet inhibition has been made, the question is how to achieve it effectively? The current evidence is discussed in this review which focuses on reversal strategies for antagonizing the antiplatelet effect of currently available oral P2Y₁₂ inhibitors clopidogrel, prasugrel, and ticagrelor only.

There is no medical need for reversal strategies for the parenteral P2Y₁₂ receptor cangrelor due to the rapid decline of platelet inhibition after stopping the infusion due to the short half-life of this compound¹⁹ (see ►Table 1).

Pharmacological Differences between Oral P2Y₁₂ Receptor Inhibitors

►Table 1 summarizes the key pharmacological and pharmacokinetic data of the currently approved P2Y₁₂ receptor inhibitors. There are substantial differences between the thienopyridine-type P2Y₁₂ platelet inhibitors (clopidogrel, prasugrel) and the nonthienopyridine derivatives ticagrelor and cangrelor.

The parent compounds clopidogrel and prasugrel are prodrugs without any antiplatelet pharmacological effect and both compounds require metabolism to the active antiplatelet compound. Clopidogrel is activated via a two-step metabolism process in the liver with the intermediate metabolite being generated by CYP1A2, CYP2B6, and CYP2C19 and further metabolism catalyzed by four enzymes (CYP2B6, CYP2C9, CYP2C19, and CYP3A4).

The more consistent and faster metabolic formation of the active metabolite (AM) of prasugrel is due to the fact that the first metabolite is formed by esterases (human carboxylesterase 2) in the gut and plasma, followed by a single cytochrome (CYP)-dependent step with primary involvement of CYP3A

Table 1 Clinical pharmacology of P2Y₁₂ receptor inhibitors

Property	Clopidogrel	Prasugrel	Ticagrelor	Cangrelor
Receptor blockade	Irreversible	Irreversible	Reversible	Reversible
Prodrug	Yes	Yes	No	No
Administration route	Oral – Once daily	Oral – Once daily	Oral – Twice daily	IV – Bolus and infusion
Half-life of parent drug	~6 hours	< 5 minutes	6–12 hours	3–9 minutes
Half-life of active metabolite	30 minutes	Distribution half-life: 30–60 minutes Elimination half-life: 2–15 hours	8–12 hours	N/A
Binding site	ADP binding site	ADP binding site	Allosteric binding site	Allosteric binding site
Mode of antagonism plasma protein	Noncompetitive	Noncompetitive	Noncompetitive	Semicompetitive
Plasma protein binding	C 98% C-AM 94%	P > 98%	T 99.8% T-AM 99.8%	n.r.
Onset of action	2–8 hours	30 minutes to 4 hours	30 minute to 4 hours	~2 minutes
Offset of action	5–10 days	7–10 days	3–5 days	60 minutes
CYP drug interaction	CYP2C19	No	CYP3A4 to T-AM	No
Approved settings	ACS (invasively / noninvasively managed), stable CAD PCI, PAD, and ischemic stroke	PCI in patients with ACS	ACS (invasively or noninvasively managed) or history of MI	PCI in patients with or without ACS

Abbreviations: ACS, acute coronary syndrome; ADP, adenosine diphosphate; AM, active metabolite; CAD, coronary artery disease; IV, intravenous; PAD, peripheral arterial disease; PCI, percutaneous coronary intervention; TAM, ticagrelor's active metabolite.

Source: Table modified from Sillén et al and Ferri et al.^{20,21}

and CYP2B6, and only partial contribution of CYP2C9 and CYP2C19. Furthermore, the pharmacological profiles of the AMs of both drugs are characterized by two distinct features: (1) they inhibit the platelet P2Y₁₂ receptor irreversibly, that is, platelet inhibition persists for the life-span of the platelet and (2) their half-lives are in the order of 30 minutes (clopidogrel) and 7 hours (prasugrel).²⁰

In contrast, the cyclopentyl-triazolo-pyrimidine derivative ticagrelor is an adenosine triphosphate (ATP) analogue with the parent compound binding reversibly in a noncompetitive manner to the P2Y₁₂ receptor at distinct sites to adenosine diphosphate (ADP) and the thienopyridines. Ticagrelor undergoes CYP3A4-mediated metabolism to an AM (AR-C124910XX), and the metabolite inhibits also the P2Y₁₂ receptor in a reversible manner. Thus, in the case of ticagrelor, half-life of elimination of parent drug and the AM (which both are in the range of 6–12 hours) determine the recovery of platelet function after cessation of drug administration. Furthermore, both ticagrelor and its AM AR-C124910XX are highly bound to plasma proteins (> 99.8%), that is, unbound fraction is less than 0.2%.²¹

Effect of Platelet Supplementation on Restoration of Platelet Function

Based upon the lack of a specific antidote for reversal of platelet inhibition by the currently used P2Y₁₂ inhibitors, a variety of in vitro and in vivo experiments were performed

aiming to assess the effect of noninhibited platelet supplementation on platelet reactivity.

Clopidogrel

Vilahrur et al administered loading doses of aspirin (325 mg) and clopidogrel (300–600 mg) to healthy subjects followed by short-term maintenance dosing with aspirin (81 mg/day) and clopidogrel (75 mg/day).²² Platelet-rich plasma (PRP) was prepared from blood samples drawn at 4 and 72 hours after starting treatment with the latter time point corresponding to 24 hours after ingestion of the last maintenance dose of both antiplatelets. Platelet inhibition by aspirin/clopidogrel was determined by agonist-induced light transmission aggregometry (LTA) and flow cytometric analysis of glycoprotein IIb/IIIa receptor expression using various stimulants. To normalize platelet reactivity in PRP from the treated subjects, increasing amounts of pooled platelets (PPs) from five untreated volunteers were added ex vivo to the PRP obtained from the treated subjects. Addition of 40 to 50% of PRP from untreated subjects normalized platelet reactivity assessed after stimulation with 10 μM ADP in LTA and further addition of 10% untreated platelets fully normalized aggregation. The authors estimated that transfusion of 10 platelet concentrate units (which was deemed equivalent to in vitro addition of 40% untreated PRP) after a 300-mg clopidogrel loading or 12.5 units (50% untreated PRP) after a 600-mg loading may adequately normalize clopidogrel-induced platelet inhibition.

The transfer of the amount of platelet concentrates required for restoration of platelet function in patients on DAPT with aspirin and clopidogrel estimated in ex vivo experiments into clinical practice is challenged by results from a study using a design which is closer to the clinical setting.²³ Prüller et al investigated in healthy subjects if transfusion of stored autologous platelets restores low platelet reactivity on DAPT (aspirin/clopidogrel). Two autologous platelet concentrates were obtained from each study participant. Thereafter, an antiplatelet regimen was started. Loading doses of aspirin 300 mg and clopidogrel 300 mg were administered followed by maintenance daily dosing of aspirin 100 mg and clopidogrel 75 mg on days 2 and 3. Two stored autologous platelet concentrates were transfused 24 hours after last dosing of the antiplatelets on day 4. A nearly complete recovery of platelet reactivity index (PRI) determined with vasodilator-stimulated phosphoprotein phosphorylation (VASP kit, Biocytex, Marseille, France) was observed after transfusion of the second platelet concentrate with a slight attenuation of effect 24 hours thereafter. However, assessment of platelet reactivity by LTA after stimulation with arachidonic acid and ADP shows hardly no immediate response to platelet transfusion and a partial recovery of platelet reactivity not earlier than 24 hour post-transfusion.

Prasugrel

Research on platelet function normalization was extended to DAPT with the combination of aspirin and prasugrel.^{24,25}

Subjects pretreated with a single dose of aspirin 325 mg received subsequently a loading dose of prasugrel 60 mg.²⁴ The experimental setting tried to estimate the earliest time after a prasugrel loading dose when added platelets are no longer inhibited by prasugrel's AM considering the longer half-life of elimination of the AM compared with clopidogrel's AM. Serial blood samples were obtained at 2, 6, 12, and 24 hours postprasugrel for platelet reactivity after stimulation with 20 μ M ADP by LTA and VerifyNow PRUtest (Werfen, Barcelona, Spain) and simultaneous determination of the plasma concentration of the AM of prasugrel. At each time point, fresh concentrated platelets from untreated donors were added ex vivo to the blood samples from treated subjects. The protocol aimed to raise the platelet counts by 0 (control), 40, 60, and 80%. Concentration-dependent increases in platelet reactivity versus respective controls by both LTA and VerifyNow PRUtest were observed after in vitro supplementation with untreated donor platelets at each time point investigated (► Fig. 1). The augmented effect at 2 hours after the prasugrel loading dose was attributed to the still high plasma concentration of the AM of prasugrel at that time (42.4 ± 11 ng/mL). A sharp increase in platelet reactivity by platelet supplementation was observed from 2 to 6 hours after loading and the effect was more or less stable thereafter. It seems that the plasma concentrations of the AM of prasugrel at 6 hours (4.5 ± 1 ng/mL) are below the threshold for a detectable inhibition of the supplemented donor platelets. Thus, administration of platelet concentrates is most effective beyond 6 hours after the last administration of prasugrel, although partial normalization of prasugrel effects could be obtained earlier.²⁴

Ticagrelor

Hobl et al administered a loading dose of ticagrelor 180 mg to healthy subjects.²⁶ Blood samples withdrawn 3 hours after dosing were spiked with autologous PRP prepared from blood collected before ticagrelor. Mixing increasing amounts of PRP with the post-dosing blood samples in ratios between 1:10 and 1:3 improved ex vivo determined ADP-induced platelet aggregation in a dose-dependent manner with marked interindividual variability. However, the predefined cut-off for restoration of platelet aggregation was achieved even at the highest platelet supplementation in 8 out of 20 subjects only.

Investigations in blood samples drawn 2 hours after dosing from patients on chronic DAPT with aspirin and ticagrelor confirmed a dose-dependent recovery of ADP-induced aggregation by increasing ex vivo platelet supplementation but it was less effective than in patients on aspirin plus clopidogrel.²⁷

The most comprehensive study performed repeated blood sampling (4, 6, 24, and 48 hour after last dosing) in patients with cardiovascular disease after single bolus dosing of ticagrelor 180 mg and aspirin 325 mg as well as after maintenance treatment with ticagrelor 90 mg twice daily and aspirin 81 mg once daily.²⁸ Patients' blood samples were supplemented with concentrated platelets from healthy donors in vitro aiming to raise platelet counts by 0 (control), 25, 50, and 75%. The time course in decline of platelet inhibition was similar after loading as well as after cessation of maintenance therapy. Platelet supplementation restored platelet reactivity in a dose-dependent manner with the restoration of function being strongly dependent on the time elapsed since last dose administration. A small but statistically significant effect could be observed as early as 6 hours after last dosing, but the clinical relevance of this improvement seems questionable. Beyond 24 hours after last dosing, platelet transfusions (2–3 units) can be expected to substantially reverse the antiplatelet effect of ticagrelor.^{28,29}

The time window between the last dose of the antiplatelet and blood sampling for assessment of the effect of platelet supplementation for reversal of platelet inhibition is of special interest for studies with ticagrelor due to the reversibility of the binding to the P2Y₁₂ receptor, the long half-lives of parent compound and the AM in plasma, and the twice daily dosing of ticagrelor in maintenance treatment. This has been shown in a study, which exposes blood from untreated healthy volunteers with blood and plasma from patients on treatment with clopidogrel, prasugrel, or ticagrelor, respectively.³⁰ Blood was drawn from the patients 3 hours after the dosing of the antiplatelets. When platelets from untreated subjects were stimulated after mixing with PRP from patients treated with different P2Y₁₂ inhibitors, ADP-induced expression of P-selectin was not affected by PRP from clopidogrel- or prasugrel-treated patients, while addition of PRP from ticagrelor-treated patients decreased surface expression of P-selectin. If blood from healthy subjects was spiked with plasma from patients on P2Y₁₂ inhibitors, ADP-induced aggregation in multiple electrode aggregometry (MEA; Multiplate, Roche Diagnostics, Rotkreuz, Switzerland) was not affected by plasma from patients on clopidogrel, was slightly inhibited by plasma

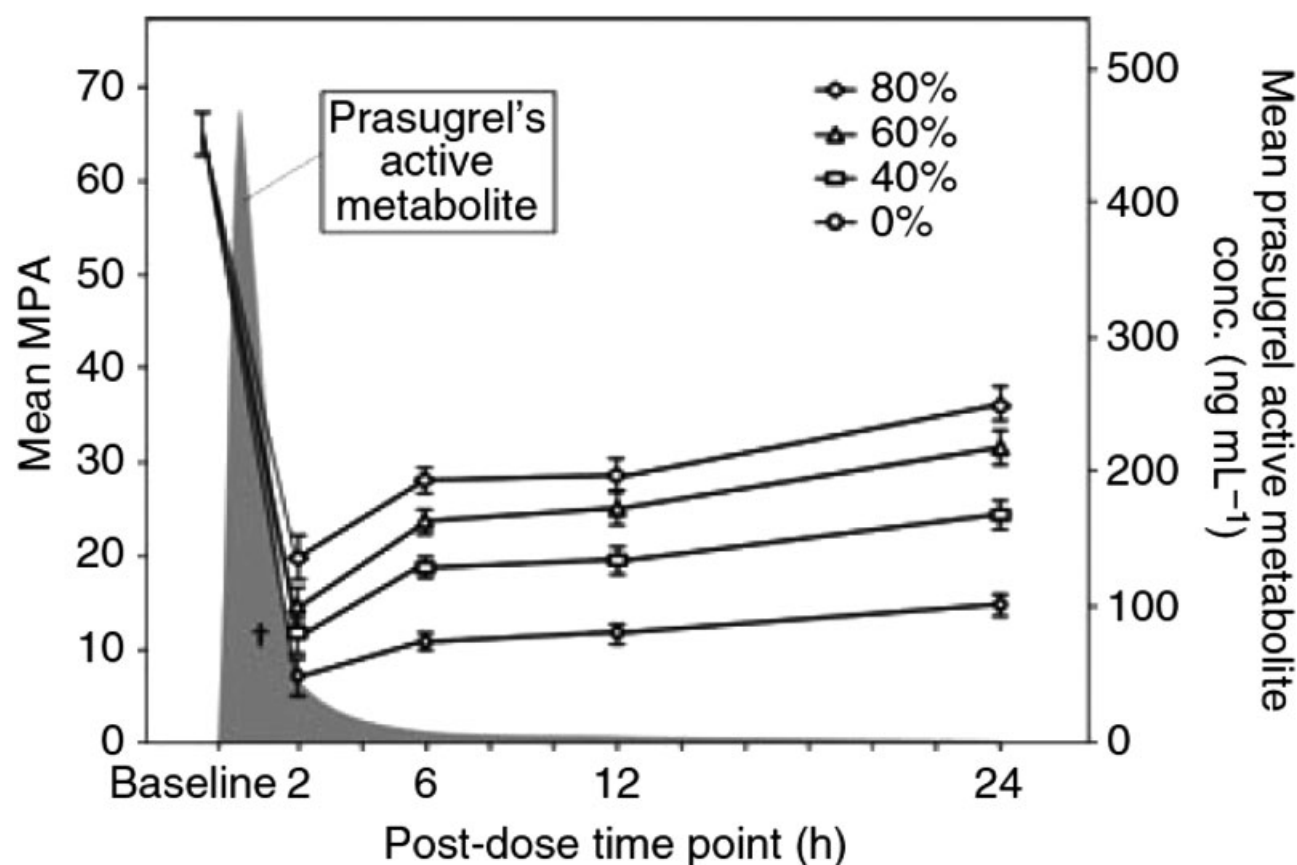


Fig. 1 Maximum platelet aggregation (MPA) to 20 μ M adenosine diphosphate (ADP) using light transmission aggregometry (LTA) (left Y-axis) and prasugrel active metabolite concentrations (right Y-axis, gray area) over 24 hours following a loading dose of prasugrel 60 mg. Platelet aggregation was measured in aspirin- and prasugrel-treated subjects' blood supplemented with (40, 60, and 80%) and without (0%) fresh platelets. Aggregation values in all supplemented samples are higher than respective controls (0%, $p \leq 0.01$) except "†" but lower than baseline ($p < 0.01$). Aggregation values in supplemented samples at 6 hours are higher than respective values at 2 hours ($p < 0.05$ for 40 and 60%), but similar to those at 12 hours ($p = \text{NS}$ for all). Prasugrel active metabolite was not measured before the 2-hour time point; results are taken from an earlier study.⁴² Figure reproduced with permission from Zafar et al.²⁴

from patients on prasugrel, and aggregation was markedly attenuated if plasma from ticagrelor patients was added. The results are in line with the expected plasma concentrations of the three antiplatelets and their AMs extrapolated from dosing regimens used and the time of blood sampling after dosing.

Schoener et al extended the reversal strategies for P2Y₁₂-related platelet inhibition based upon platelet supplementation by an interesting approach considering the high plasma protein binding of ticagrelor and its AM.³¹ Patients on DAPT (aspirin plus guideline-recommended dosing with clopidogrel, prasugrel, or ticagrelor) with an ACS were enrolled. Blood samples were drawn at trough of the respective chronic dosing regimen and not earlier than 24 hours after loading. Platelet reactivity was assessed by VASP test. Supplementation with increasing amounts of freshly prepared PRP from healthy donors increased VASP-PRI irrespective of the antiplatelet drug administered (**Fig. 2**). PPs obtained from the local blood bank restored platelet inhibition in clopidogrel and prasugrel samples but had surprisingly no effect on samples from ticagrelor-treated patients. Further experiments with samples from ticagrelor-treated patients investigated the reversal effect of sedimented PRP platelets which were resuspended in PPs buffer. No reversal effect could be determined although the

same amounts of PRP or PP were administered (**Fig. 3**). PRP preparations contain physiological concentrations of plasma proteins, while the PP concentrates provided from the local blood bank are free of relevant amounts of protein since they are stored in a special stabilizing buffer solution. This fact indicates that proteins—namely serum albumin—might contribute to the different effects on ticagrelor reversal observed between supplementation of PRP and PP. To test this hypothesis, increasing amounts of human serum albumin in the range of 8 to 80 g/L were added to samples from ticagrelor-treated patients. Supplementation with serum albumin restored platelet inhibition by ticagrelor since VASP-PRI increased in a concentration-dependent manner (**Fig. 3**). Similar investigations with the same concentration range of human serum albumin were performed with samples from clopidogrel- as well as prasugrel-treated patients. Platelet inhibition by clopidogrel and prasugrel was reversed less pronounced by supplementation of human serum albumin which might be attributed to the irreversible mode of receptor binding of clopidogrel and prasugrel. Thus, the approach of albumin supplementation for reversal of ticagrelor-induced platelet inhibition might be a possible and clinically applicable approach. The in vitro added concentrations of human serum

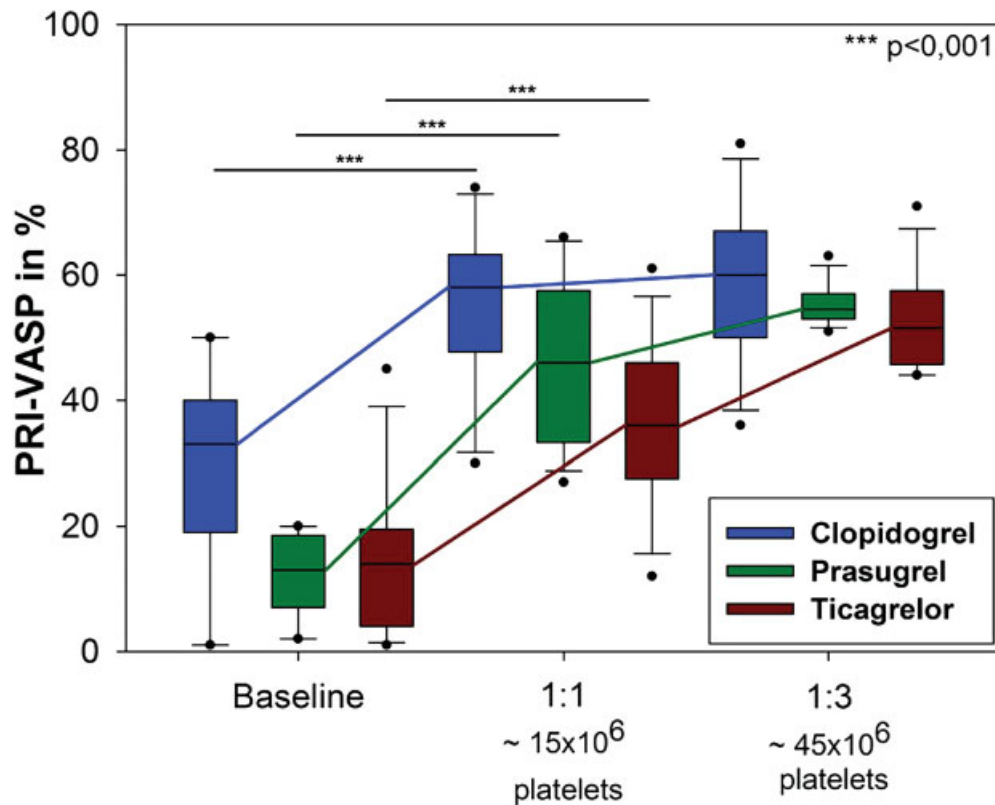


Fig. 2 Reversal of platelet inhibition by clopidogrel, prasugrel, and ticagrelor determined by vasodilator-stimulated phosphoprotein-platelet reactivity index (VASP-PRI) after ex vivo platelet supplementation using drug-free platelet-rich plasma (PRP). Figure reproduced with permission from Schoener et al.³¹

album correspond to doses of approximately 20 to 40 g human albumin in vivo. However, clinical data in patients with acute severe bleeding or patients scheduled for urgent surgical procedures are lacking so far.

The Action Study Group performed the APTITUDE study (Efficacy of Ex Vivo Autologous and In Vivo Platelet Transfusion in the Reversal of P2Y₁₂ Inhibition by Clopidogrel, Prasugrel, and Ticagrelor).³² APTITUDE-ACS enrolled patients presenting with an ACS or for elective PCI. Normalization of P2Y₁₂ inhibition by clopidogrel, prasugrel, and ticagrelor was investigated by ex vivo supplementation of autologous platelets. In their ex vivo experiments, PRP from treatment-naïve subjects was mixed in increasing proportions (30, 50, and 80%) with PRP sampled 4 hours after loading doses of either clopidogrel 600 mg, clopidogrel 900 mg, prasugrel 60 mg, or ticagrelor 180 mg. In the in vitro experiments, the percentage restoration of residual platelet aggregation (assessed by LTA after stimulation with 20 µM ADP) increased with increasing amounts of supplemented treatment-naïve platelets. The finally achieved platelet reactivity obtained with 80% proportion supplemented platelets decreased with increasing potency of the P2Y₁₂ receptor blocking intensity in the order clopidogrel 600 mg < clopidogrel 900 mg < prasugrel 60 mg < ticagrelor 180 mg.

Patients with excessive bleeding undergoing cardiac surgery while on a maintenance dose of aspirin plus either clopidogrel, prasugrel, or ticagrelor were enrolled into the APTITUDE-Coronary Artery Bypass Graft (APTITUDE-CABG) substudy.³² Platelet reactivity was assessed by VASP reactivity

index before and within 30 minutes after completion of the transfusion of platelet concentrates. The dose of the platelet concentrates was set according to a weight-based guideline (mean 5.5 concentrate units of platelets). While a statistically significant increase in VASP reactivity index (relative increase 23.1%) was observed in patients on clopidogrel, only a small nonsignificant increase was observed in the patients on prasugrel/ticagrelor. Although the number of patients undergoing surgery on prasugrel/ticagrelor was small, one might speculate that the attenuated efficacy of the platelet transfusions might be attributed at least in part to the use of pooled buffy coat platelets in the majority of patients.³¹

A case report highlights the challenges in management of patients with life-threatening bleeding on DAPT with ticagrelor.³³ A 65-year-old man on DAPT with aspirin and ticagrelor post-PCI with stent implantation was admitted with hemiplegia and hemispatial neglect. Twelve hours after thrombolysis with recombinant tissue plasminogen activator consciousness of the patient decreased and an intracranial hematoma with intraventricular hemorrhage and acute hydrocephalus requiring an external ventricular drain was diagnosed. The patient died shortly after surgery despite transfusion of 17 platelet concentrates aiming to reverse the effects of antiplatelet therapy. Platelet function assays performed before and after transfusion of platelet concentrates indicate significant attenuation of arachidonic acid-induced aggregation (VerifyNow Aspirin test), while no reversal of P2Y₁₂ inhibition could be demonstrated neither by VerifyNow PRUtest nor by VASP-PRI.

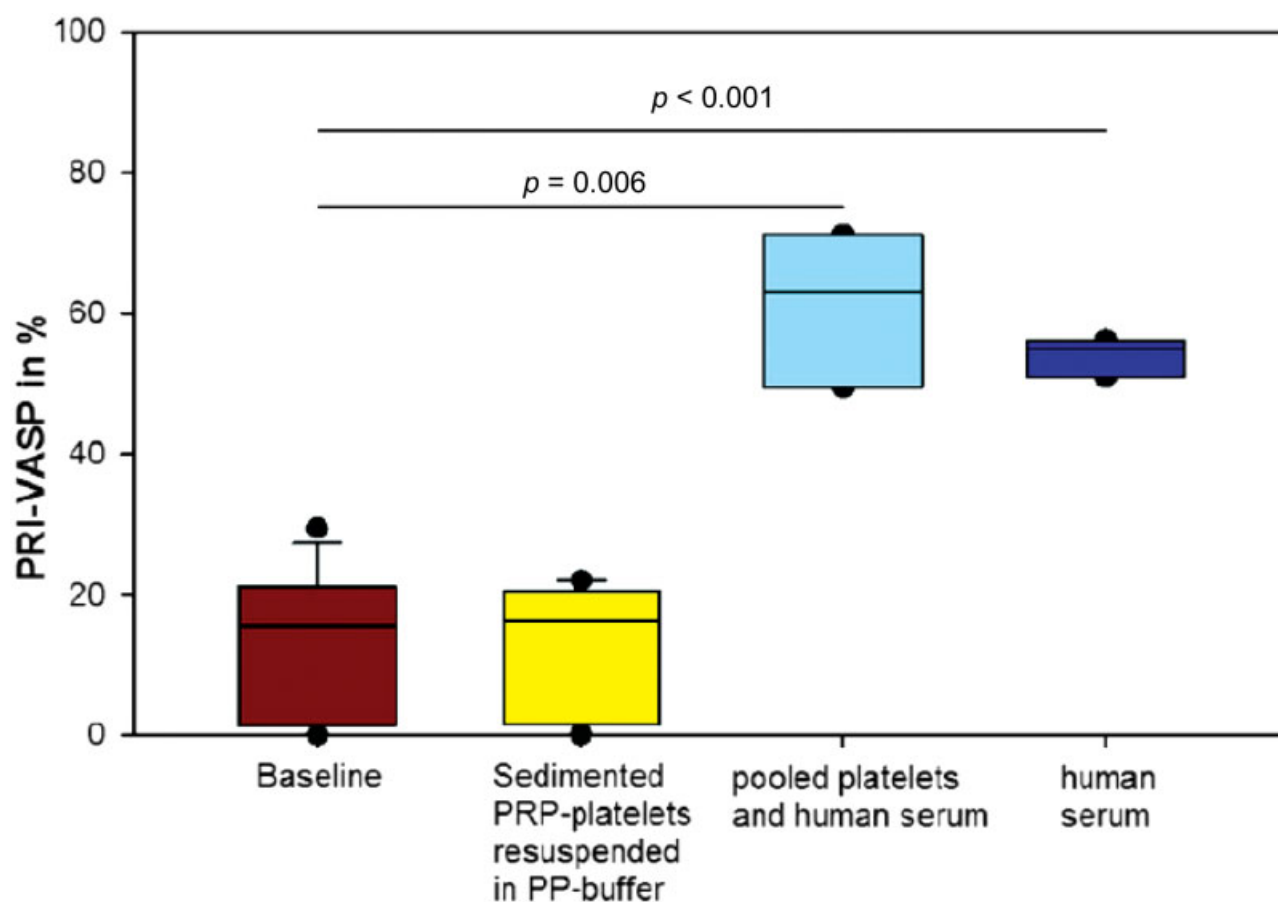


Fig. 3 Change in vasodilator-stimulated phosphoprotein-platelet reactivity index (VASP-PRI) after supplementation of platelet-rich plasma (PRP) platelets resuspended in pooled platelets (PPs) buffer, and PP with human serum and human serum alone in ticagrelor-treated patients. A significant VASP increase after adding of human serum with or without PP is evident. Figure reproduced with permission from Schoener et al.³¹

Hemadsorption for Reversal of Ticagrelor

Only in vitro data on the use of sorbent hemadsorption as an alternate method for removal of ticagrelor from blood are available so far.³⁴ The styrene copolymer Porapak Q 50–80 mesh and CytoSorb were used to investigate the removal of ticagrelor from bovine serum albumin solutions, whereas CytoSorb was also used to remove the drug from human blood samples (►Fig. 4). The hemadsorption technique was highly effective in removing the drug ticagrelor, but no functional data on reversal of platelet inhibition are available so far. Besides this, several questions remain to be investigated at present. These comprise the efficiency of removal of the AM of ticagrelor as well as the suitability of the method for emergent situations when a slow removal is clinically unacceptable. Furthermore, the effect of hemadsorption on untended removal of other drugs or endogenous compounds needs to be investigated.

Human Monoclonal Antigen-Binding Fragment PB2452 for Reversal of Ticagrelor

PB2452 (former name MEDI2452) is an antigen-binding fragment (Fab) antidote for ticagrelor.³⁵ The Fab has a 20 pM affinity for ticagrelor, which is 100 times stronger than ticagrelor's affinity for the platelet P2Y₁₂ receptor. The

binding characteristics for ticagrelor's AM (TAM) are similar. PB2452 is highly specific for ticagrelor/TAM and pre-clinical experiments excluded binding to adenosine, ATP, ADP, or a variety of structurally related drugs. The antidote neutralizes in a concentration-dependent manner free (unbound) ticagrelor/TAM in plasma (►Fig. 5), causes a dissociation of both compounds from the P2Y₁₂ receptor to the plasma compartment enabling binding by the circulating Fab. This results in a PB2452 concentration-dependent reversal of ticagrelor/TAM-induced inhibition of human platelets (►Fig. 5). The Fab reverses ticagrelor-induced bleeding in a mice tail bleeding model. It was assumed from the preclinical data, that the amount of ticagrelor/TAM in a patient is neutralized completely within minutes after administration of PB2452.³⁶

The further preclinical assessment included investigation of the hemostatic effect of PB2452 in a pig bleeding model after treatment of the animals with aspirin and ticagrelor.³⁷ Administration of PB2452 completely neutralized free ticagrelor/TAM within 5 minutes after administration of the Fab which translates into a gradual normalization of ADP-induced platelet aggregation and a numerical reduction in blood loss.

Most recently, the results of a first randomized, double-blind, placebo-controlled phase 1 trial for assessment of dose-finding, safety, efficacy, and pharmacokinetics of PB2425 in healthy subjects pretreated with ticagrelor were published.³⁸

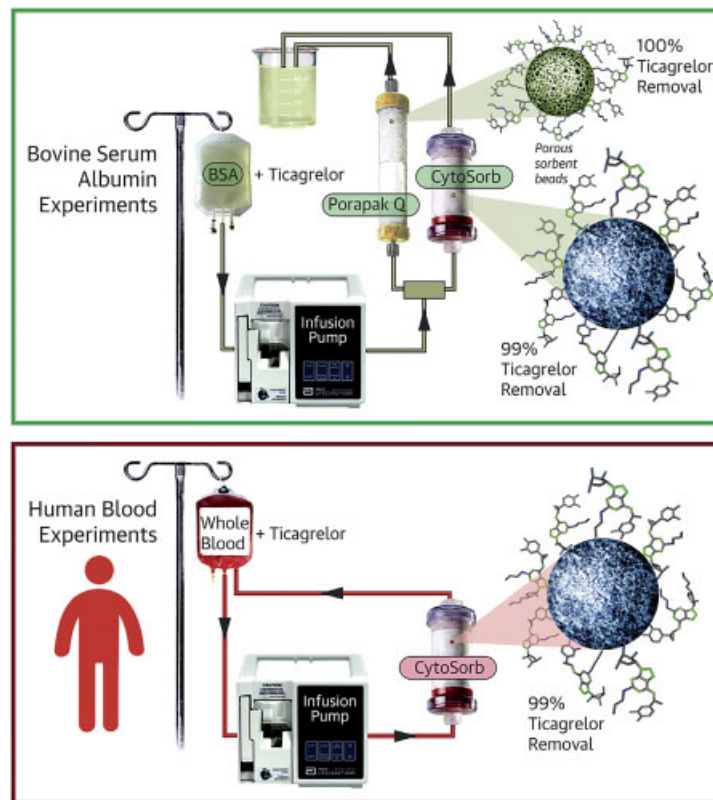


Fig. 4 Illustration of the experimental ex vivo setting of sorbent hemadsorption for ticagrelor removal. The upper panel illustrates the first-pass experiment using bovine serum albumin (BSA) solution preincubated with ticagrelor. The lower panel displays the recirculating technique used for human blood spiked with ticagrelor. Figure reproduced with permission from Angheloiu et al.³⁴

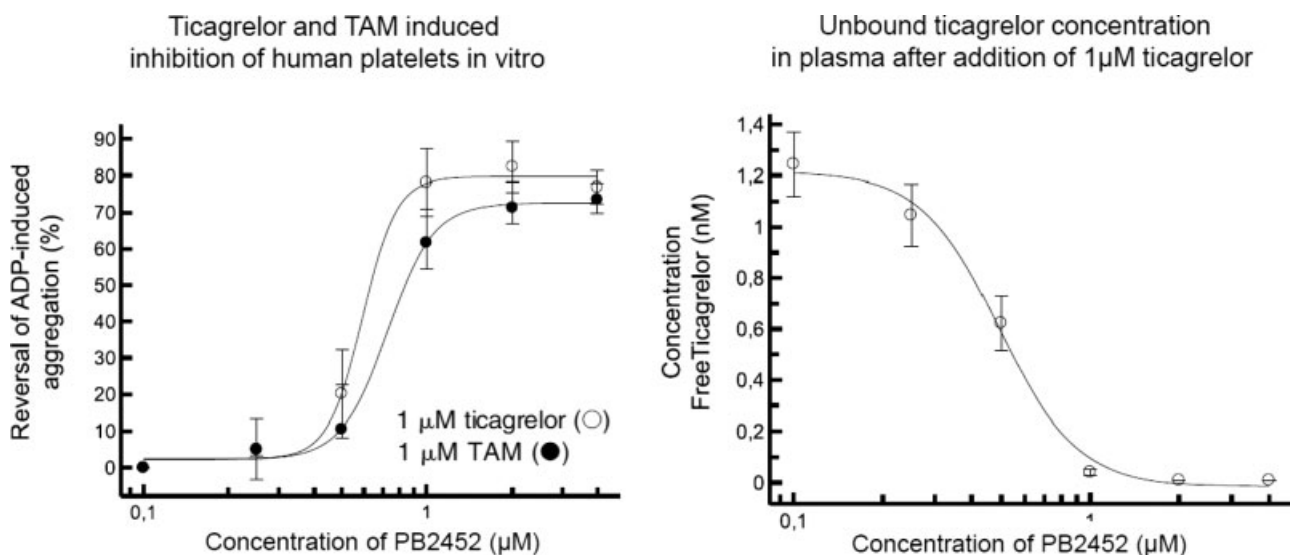


Fig. 5 In vitro concentration-response curves of the Fab fragment PB2452 on platelet inhibition by ticagrelor and the active metabolite of ticagrelor (left panel) and the reduction of unbound ticagrelor concentration (right panel). Figures adapted from Buchanan et al.³⁵

Ten sequential dose cohorts of PB2452 were evaluated. The antibody was administered either as a 30-minute infusion (doses 0.1–9 g) or by a bolus infusion followed by a prolonged infusion for between 8 and up to 16 hours (total dose of 18 g). Platelet function was assessed using LTA after stimulation with 20 (5) μM ADP, VerifyNow PRUtest, and VASP assays before and up to 48 hours after ticagrelor administration.

Subjects were pre-treated for 48 hours with ticagrelor (loading dose of 180 mg followed by 90 mg twice daily). A rapid but transient reversal of ticagrelor-induced platelet inhibition with 3 and 9 g of PB2452 was observed in LTA with a dose-dependent duration of reversal lasting 2 hours with the 9 g infusion. Following the bolus plus prolonged infusion regimen of 18 g PB2452, a significant reversal was observed

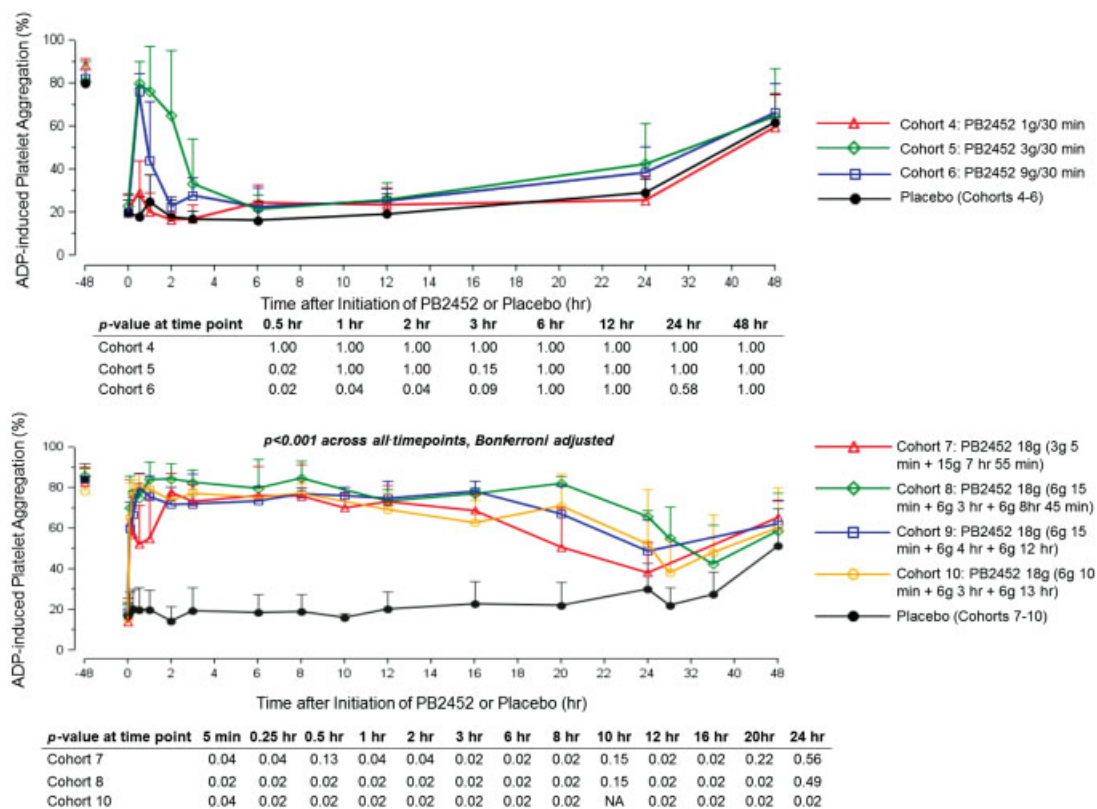


Fig. 6 Time course of the onset and offset of ticagrelor reversal by adenosine diphosphate (ADP)-induced platelet aggregation after administration of the monoclonal antibody fragment PB2452 to healthy subjects after pretreatment with ticagrelor. (A) Effect of ascending doses of PB2452 or placebo administered as a 30-minute short-term infusion. (B) Effect of an intravenous infusion of 18 g PB2452 or placebo administered with an infusion duration of 8, 12, or 16 hours. Figures adapted from Bhatt et al.³⁸

5 minutes after initiation of PB2452 infusion (→Fig. 6). The duration of reversal was infusion time-dependent, lasting 20 to 24 hours with a 16-hour infusion. There was no evidence of rebound in platelet activity after cessation of PB2452. Data were consistent throughout all platelet function assays used. Adverse events related to the Fab fragment were limited mainly to issues involving the infusion site. Thus, administration of the antibody PB2452 is at present the most rapid and effective reversal strategy for the antiplatelet effect of ticagrelor.

The clinical efficacy for treatment or prevention of bleeding remains to be investigated in further studies. The reversal agent reduces blood loss in animal models of bleeding.³⁷ Clinical data as the effective reversal of antiplatelet effect results in a more rapid restoration of hemostasis in patients bleeding on ticagrelor or the prevention of bleeding are lacking at present. If the reversal agent confirms these expectations in clinical efficacy in future studies, it would be an important advance.

Conclusion

The beneficial effect of DAPT concerning reduction of risk of ischemic events such as myocardial infarction or stent thrombosis has been shown in numerous clinical studies. In the year 2017, the concept of DAPT—which established its superiority over anticoagulant therapy among patients un-

dergoing PCI—has its 21st anniversary since the publication of the first randomized clinical trial (ISAR Trial⁵). Based on over 35 randomized clinical trials, including more than 225,000 patients, DAPT is among the most intensively investigated treatment options in the field of cardiovascular medicine.¹⁷ The vast majority of studies focused primarily on the reduction of the risk of ischemic events including (re)-infarction and stent thrombosis. On the contrary, this well-recognized improvement in ischemic benefit by DAPT is accompanied by an increased on-treatment bleeding risk including major and fatal bleeding.

Current concepts of personalized antiplatelet therapy aim to establish alternative DAPT strategies and to preserve the benefit in prevention of ischemic events while reducing on-treatment bleeding risk. TROPICAL-ACS was the first prospective randomized study with an appropriate sample size in this context demonstrating the feasibility of an early de-escalation strategy of P2Y₁₂ inhibition in ACS patients guided by platelet function testing.^{39–41}

Supplementation of platelets is the most extensively studied strategy for timely reversal of P2Y₁₂ inhibition in emergency situation such as acute major bleeding or urgent surgery with high bleeding risk or unacceptable clinical consequences of bleeding. These studies investigate recovery of ex vivo determined platelet function as a surrogate for bleeding risk. Using this approach, reversal of the thienopyridine derivatives clopidogrel and prasugrel which inhibit the

P2Y₁₂ receptor for the life-span of the platelet is feasible. Data on reduction of platelet inhibition after administration of ticagrelor which has reversible binding characteristics are conflicting. Addition of human serum albumin in vitro experiments has been shown to be an interesting alternative. Hemadsorption as an alternate approach is currently at the bench level and doubts concerning the suitability of this method in emergency situations requiring rapid reversal persist. However, besides the APTITUDE-CABG study,³² a study in healthy subjects,²³ and case reports,³³ the vast majority of the strategies to assess reversal strategies for P2Y₁₂ inhibition are ex vivo measurements investigating increase in platelet reactivity as surrogate. Proof-of-concept studies on the clinical efficacy of these strategies on restoration of hemostasis are lacking. First dose-finding data on the use of the Fab fragment PB2452 in healthy subjects were recently published.³⁸ It is at present the most promising option for reversal of ticagrelor, since platelet function is reversed rapidly and overall tolerability of the antibody seems to be without major concerns. The study provides promising findings but achieving longer periods of reversal required higher doses and longer infusion periods of PB2452.³⁸ At present, we do not have any estimate on the costs for this treatment. Since U.S. Food and Drug Administration grants breakthrough therapy designation for PB2452, clinical availability might be expected in the near future.

The available in vitro studies used appropriate laboratory assays to assess the pharmacological activity on platelet function of the P2Y₁₂ receptor antagonists and its reversal. Major concerns persist, if these methods reflect the interaction of platelets, the signaling cascades in the coagulation pathways, and the entire complex coagulation system to achieve hemostasis and to reduce blood loss in the clinical setting. Adequately powered clinical trials with bleeding outcome assessment would be required before definite clinical recommendations can be derived from these so far more or less experimental studies. It is more than doubtful if such trials will ever be conducted in an adequate setting, with enrollment of a sufficient number of patients and last but not least in a timely manner.

Conflict of Interest

D.T. reports lecture fees from Amgen, AstraZeneca, Bayer, Berlin Chemie, Boehringer Ingelheim, Bristol-Myers Squibb, Daiichi Sankyo, Pfizer, and Sanofi and fees for advisory board activities from Bayer, Boehringer Ingelheim, and Daiichi Sankyo. D.A. reports lecture fees from Astra Zeneca, Bayer, Boehringer Ingelheim, Pfizer, and MSD Pharma. K.S. reports personal fees from Bayer. D.S. reports grants and personal fees from Sanofi Aventis, Roche Diagnostics, and Daiichi Sankyo; personal fees from Bayer, Pfizer, and Haemonetics S.A. All the other authors have no conflict of interest to declare.

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