

# Platelet P2Y<sub>12</sub> receptor antagonist pharmacokinetics and pharmacodynamics: A foundation for distinguishing mechanisms of bleeding and anticipated risk for platelet-directed therapies

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## Summary

The platelet P2Y<sub>12</sub> receptor is involved in all aspects of arterial thrombosis, including adhesion, activation, aggregation, secretion and development of a stable aggregate on which coagulation proteins can assemble and fibrin strands can mesh. Inhibition of the P2Y<sub>12</sub> receptor has been shown convincingly to reduce cardiovascular events among patients with acute coronary syndromes (ACS) and in patients undergoing percutaneous intervention (PCI). Current studies are exploring whether there is a threshold of platelet aggregation below which only more

bleeding occurs, without a concomitant reduction in clinical events. The following review considers the potential relevance of reversible and irreversible mechanisms of P2Y<sub>12</sub> inhibition to bleeding risk, posing the question, "Is it not only *how much* but *how* a platelet P2Y<sub>12</sub> receptor is inhibited that determines the attributable safety profile?"

## Keywords

Platelet P2Y<sub>12</sub> receptor, clopidogrel, prasugrel, ticagrelor, cangrelor

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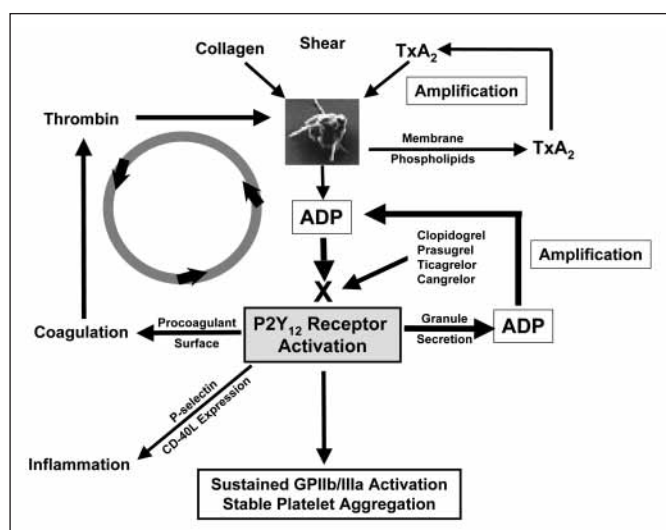
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## Introduction

Platelet inhibition with aspirin and clopidogrel constitutes a cornerstone of treatment to prevent thrombotic events in patients with acute coronary syndromes (ACS) and those undergoing percutaneous coronary intervention (PCI) (1–5). Pharmacodynamic studies have revealed various limitations of current clopidogrel therapy, including a delayed antiplatelet response; an overall modest degree of platelet inhibition (approximately 30–50%); distinct response variability, with a substantial percentage of patients exhibiting non-responsiveness; and irreversible platelet inhibition that may critically affect outcomes in patients needing urgent surgery with exposure to an unpredictable risk of bleeding and ischaemia (6, 7). Numerous attempts have been made to develop P2Y<sub>12</sub> inhibitors that overcome such drawbacks, including thienopyridines that are more efficiently metabolised than clopidogrel and direct-acting inhibitors that bind reversibly to the P2Y<sub>12</sub> receptor. The aim of this review is to define and contrast the mechanism of P2Y<sub>12</sub> inhibition by the various agents that may explain differences in bleeding and the occurrence of ischaemic events. We used Medline to search the relevant literature.

## The platelet P2Y<sub>12</sub> receptor as a target for inhibition of platelet function

The P2Y<sub>12</sub> receptor plays a central role in platelet function (► Fig. 1) (8). Platelet activation provokes numerous responses that participate in thrombosis and inflammation, including platelet aggregation, dense granule secretion of adenosine diphosphate (ADP), release of coagulation factors, and expression of activation-dependent adhesion molecules that affect leukocyte function. Co-activation of the P2Y<sub>1</sub> receptor (coupled to a G<sub>q</sub> protein, mediating phospholipase C activation and calcium mobilisation) and the P2Y<sub>12</sub> receptor (coupled to G<sub>i</sub>, mediating inhibition of adenylate cyclase) is necessary for a full ADP-induced response, with activation of the P2Y<sub>1</sub> receptor alone resulting in a modest, transient and unstable aggregation. The dense granule and alpha granule secretory response caused by ADP agonists is initiated by P2Y<sub>1</sub> activation and highly dependent on concomitant P2Y<sub>12</sub> activation for amplification. ADP-stimulated P2Y<sub>12</sub> activation of G<sub>i</sub> is central to subsequent amplification of platelet activation in response to other, more robust platelet agonists (eg, thrombin, collagen and thromboxane A<sub>2</sub>), by playing a major role in sustaining GPIIb/IIIa activation and stable platelet aggregation (9). In addition, it is involved in augmenting granule secretion, increasing expression of



**Figure 1: P2Y<sub>12</sub> is the pivotal platelet receptor.** Activation of this receptor provokes numerous responses that participate in thrombosis and inflammation, including platelet aggregation, dense granule secretion of ADP and release of coagulation factors. Activation of the P2Y<sub>12</sub> receptor by ADP and the thromboxane receptors by TxA<sub>2</sub> is also involved in transforming the GPIIb/IIIa receptors to an active state, facilitating irreversible platelet aggregation and stable clot generation. Antagonism of the P2Y<sub>12</sub> receptor with an agent such as clopidogrel, prasugrel, ticagrelor or cangrelor is expected to inhibit thrombus formation, procoagulant activity and inflammatory processes. ADP=adenosine diphosphate; CD=clusters of differentiation; GP=glycoprotein; TxA<sub>2</sub>=thromboxane A<sub>2</sub>. Adapted with permission from Gurbel et al (8).

P-selectin (which exerts a pro-inflammatory effect through interaction with leukocytes) and promoting procoagulant changes in the platelet surface membrane stimulated by other agonists. P2Y<sub>12</sub> activation also plays a role in shear-induced aggregation. Inhibition of the P2Y<sub>12</sub> receptor can thus be expected to attenuate aggregation, as well as procoagulant and inflammatory responses

that contribute to both the pathobiology and the clinical expression of coronary atherothrombosis.

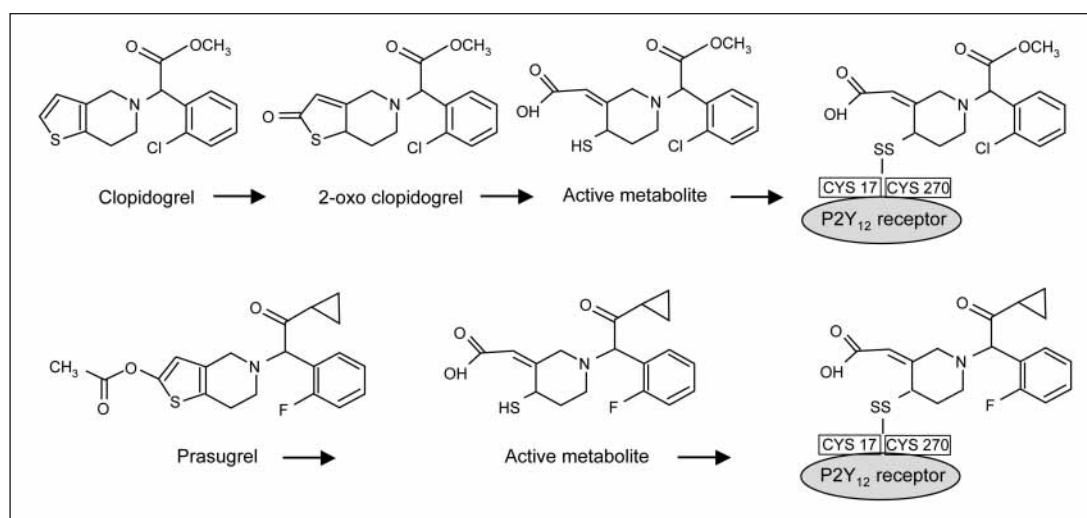
## Drug antagonists

### Thienopyridines

#### Clopidogrel

Although clopidogrel and ticlopidine have similar efficacy profiles, the superior safety profile of clopidogrel has allowed it to largely replace ticlopidine in the management of patients with coronary atherothrombosis. Both agents are orally administered prodrugs that are metabolised to an active form via the hepatic cytochrome P450 (CYP 450) isoenzymes (6, 10, 11). Clopidogrel is extensively hydrolysed by esterases, such that only 15% of the absorbed dose reaches the therapeutic CYP 450 site. In a two-step oxidation process, clopidogrel is converted initially to 2-oxo-clopidogrel and then, via hydrolysis, to a highly unstable thiol derivative; the active metabolites of both clopidogrel and ticlopidine feature a carboxylic acid and a thiol group produced upon thiophene ring opening (► Fig. 2). The clopidogrel active metabolite irreversibly and selectively inhibits the P2Y<sub>12</sub> receptor that is associated with dose-dependent blockade of ADP binding as measured by assays utilising 2MeS-ADP (► Fig. 3) (11–13). The irreversible modification of the ADP P2Y<sub>12</sub> receptor site follows the formation of a disulfide bridge between the reactive thiol group of the active metabolite and the two extracellular cysteine residues (Cys 17 and Cys 270) of the P2Y<sub>12</sub> receptor (10, 14, 15).

Studies with standard ticlopidine and clopidogrel maintenance doses reveal maximal inhibition (up to 30–50%) of ADP-induced platelet aggregation after approximately 5 days (15–18). The irreversible binding of the active metabolite causes persistent inhibitory action for several days after dosing, consistent with the 7- to 10-day platelet life span. Accordingly, restoration of normal



**Figure 2: Chemical structures of clopidogrel, prasugrel and their active metabolites.** Adapted with permission from Savi et al., Sugidachi et al. (10, 25).

functionality is dependent on the introduction of new platelets into the circulation. The irreversible inhibition of the ADP receptor and the long recovery time span leads to a heightened risk of bleeding during surgical procedures, prompting a recommendation that treatment be stopped at least 5 days before surgery (19–21).

### Prasugrel

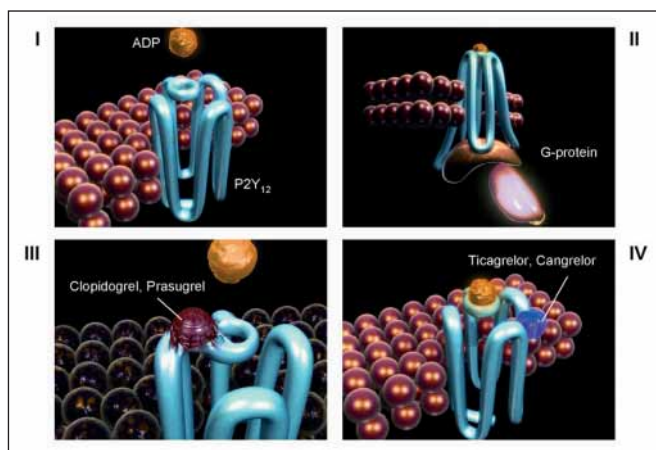
Prasugrel is an oral third-generation thienopyridine prodrug that produces rapid and robust platelet inhibition due to highly efficient metabolism to the active metabolite (22–24). Prasugrel is rapidly hydrolysed by carboxylesterases to a thiolactone, which is then efficiently converted to the active derivative via CYP isoenzymes in a one-step process. The active form binds covalently to the P2Y<sub>12</sub> receptor via a disulfide bond similar to that formed by the clopidogrel active metabolite, permanently blocking the receptor for ADP binding (Figs. 2, 3) (23, 25).

The hypothesis that more rapid achievement and higher levels of platelet aggregation inhibition would produce better clinical outcomes was indirectly assessed in the phase III Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel-Thrombolysis in Myocardial Infarction 38 (TRITON-TIMI 38), where prasugrel (60 mg loading dose, 10 mg daily maintenance dose) treatment was compared with clopidogrel (300 mg loading dose, 75 mg daily maintenance) in patients with acute coronary syndromes undergoing PCI (26). Prasugrel treatment was associated with a significant 19% relative reduction in risk for the primary endpoint (cardiovascular death, non-fatal myocardial infarction, stroke), as well as significant reductions in risk for myocardial infarction and urgent target vessel revascularisation. However, the occurrence of major bleeding, both life-threatening bleeding and fatal bleeding were also significantly increased with prasugrel treatment. It should be noted that clinical events potentially indicating treatment failure occurred in 10% of treated patients, and it is possible that a ceiling effect of P2Y<sub>12</sub> blockade using an irreversible agent in attenuating the occurrence of ischaemic events was observed in the TRITON-TIMI 38 trial. Since a platelet function sub-study of significant size was not conducted within the trial, it is not possible to determine whether treatment failure with prasugrel was associated with high on-treatment platelet reactivity (discussed in greater detail below). The occurrence of significant bleeding in the TRITON trial may also be a feature of robust but irreversible P2Y<sub>12</sub> blockade.

### Reversibly binding P2Y<sub>12</sub> receptor antagonists

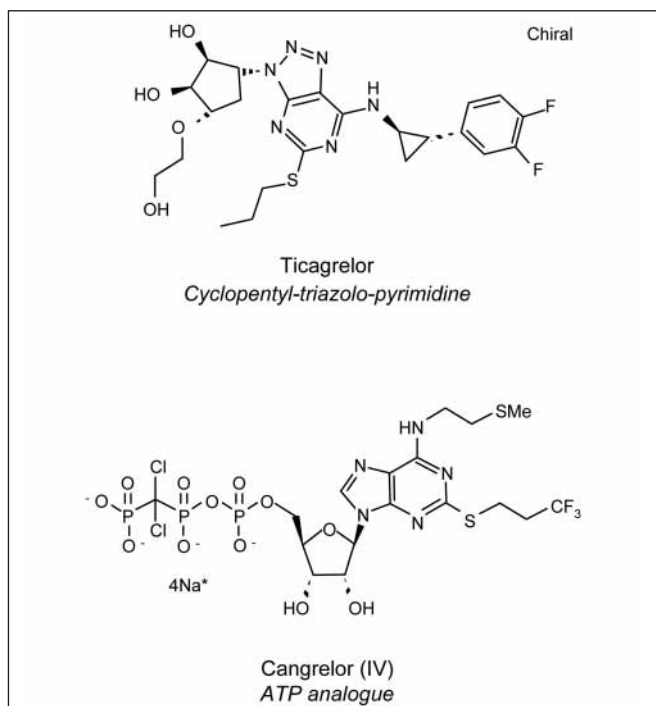
#### Ticagrelor

Ticagrelor (formerly known as AZD6140), a reversibly binding oral P2Y<sub>12</sub> receptor antagonist, is the first of the new cyclopentyl-triazolo-pyrimidine chemical class of antiplatelet agents (27–30). Unlike cangrelor (see below), ticagrelor is not an ATP analogue and does not contain the triphosphate moiety that is susceptible to hy-

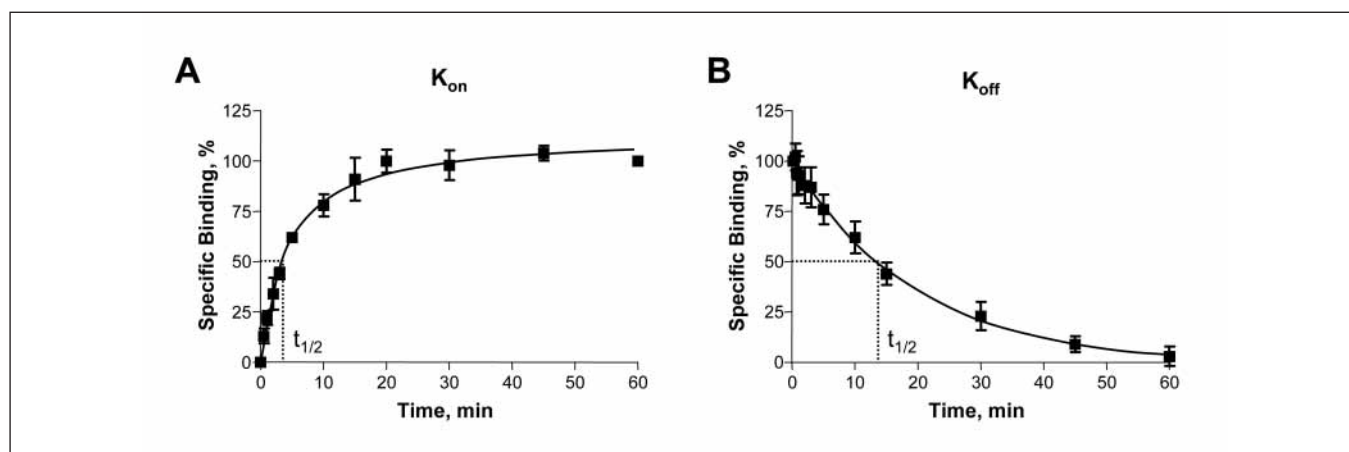


**Figure 3: Binding of irreversible and reversible P2Y<sub>12</sub> receptor antagonists.** I, II) ADP binds to the P2Y<sub>12</sub> receptor, resulting in a conformational change and G-protein-coupled activation. III) The active metabolites of clopidogrel and prasugrel bind irreversibly to the P2Y<sub>12</sub> receptor, deactivating it for the life span of the platelet. IV) Ticagrelor and cangrelor bind reversibly to P2Y<sub>12</sub> at a site distinct from that occupied by ADP; reversible antagonism occurs as these agents transform the receptor to an inactive state. Upon dissociation of the reversible agents from this site, the P2Y<sub>12</sub> receptor regains its function. Adapted with permission from Husted et al. (34).

drolisis (► Fig. 4). The drug acts directly on the P2Y<sub>12</sub> receptor and does not require metabolic activation, even though one of the circulating metabolites also has a P2Y<sub>12</sub> inhibiting activity comparable to the parent compound (30). At therapeutic doses, ti-



**Figure 4: Chemical structures of ticagrelor and cangrelor.** Reproduced with permission from van Giezen et al. (31).

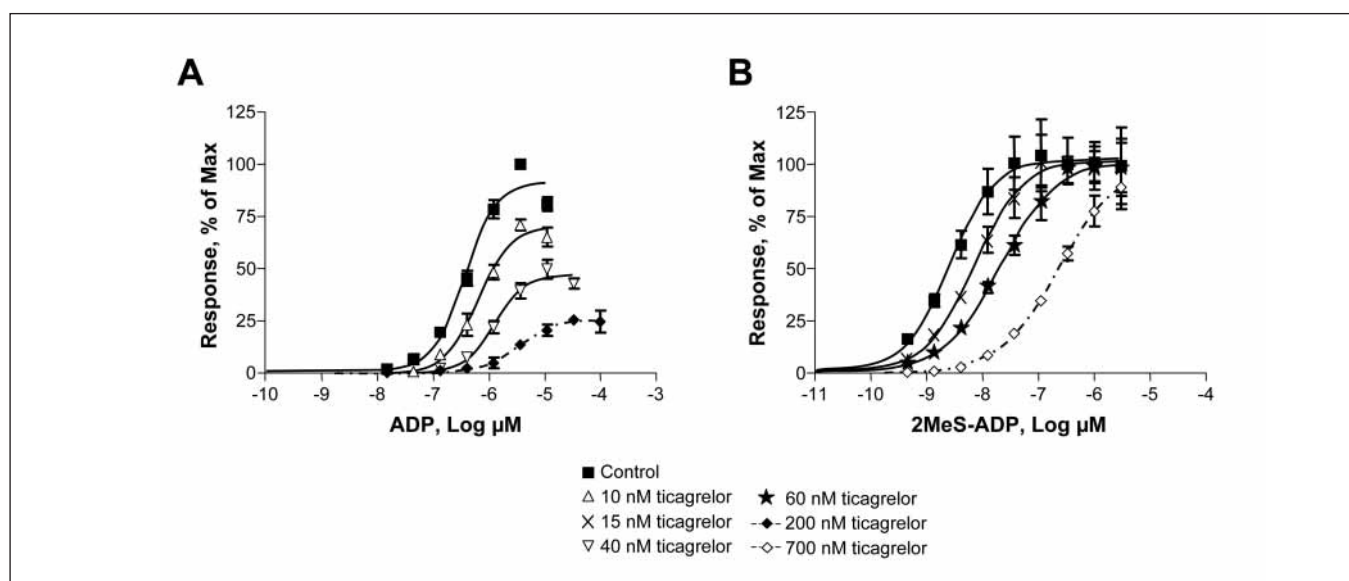


**Figure 5: Determination of the  $K_{on}$  (A) and  $K_{off}$  (B) for  $^3\text{H}$ -ticagrelor binding to the human P2Y<sub>12</sub> receptor expressed in CHO-K1 cells.**  $^3\text{H}$ -ticagrelor binds reversibly to the human P2Y<sub>12</sub> receptor with a  $K_{on}$  and  $K_{off}$  of  $1.1 \pm 0.2 \times 10^{-4} \text{ nM}^{-1} \text{ s}^{-1}$  and  $8.7 \pm 1.4 \times 10^{-4} \text{ s}^{-1}$ , respectively. Times to achieve 50% binding ( $t_{1/2(on)}$ ) or 50% dissociation ( $t_{1/2(off)}$ ) were  $3.8 \pm 0.9 \text{ min}$  and  $13.5 \pm 1.9 \text{ min}$ , respectively. Reproduced with permission from van Giezen et al. (32).

ticagrelor rapidly produces potent inhibition of ADP-mediated platelet aggregation, with inhibitory activity correlating closely with plasma drug concentrations (27, 28, 31). The reversible binding of ticagrelor was demonstrated in studies of rh-P2Y<sub>12</sub> receptor-transfected CHO-K1 cells, showing that the compound exhibited a potent, rapid and reversible binding (equilibrium  $K_d = 10.5 \text{ nM}$ ,  $k_{on} = 0.00011 \text{ nM}^{-1} \text{ s}^{-1}$  and  $k_{off} = 0.00087 \text{ s}^{-1}$ ), with half-life values of approximately 4 minutes (min) for binding and 14 min for unbinding (► Fig. 5) (32). These receptor binding data are in line with the observed rapid recovery of platelet function observed in a single ascending dose study in healthy volunteers (33). Additional studies suggest that ticagrelor binds to the P2Y<sub>12</sub> receptor at a site different

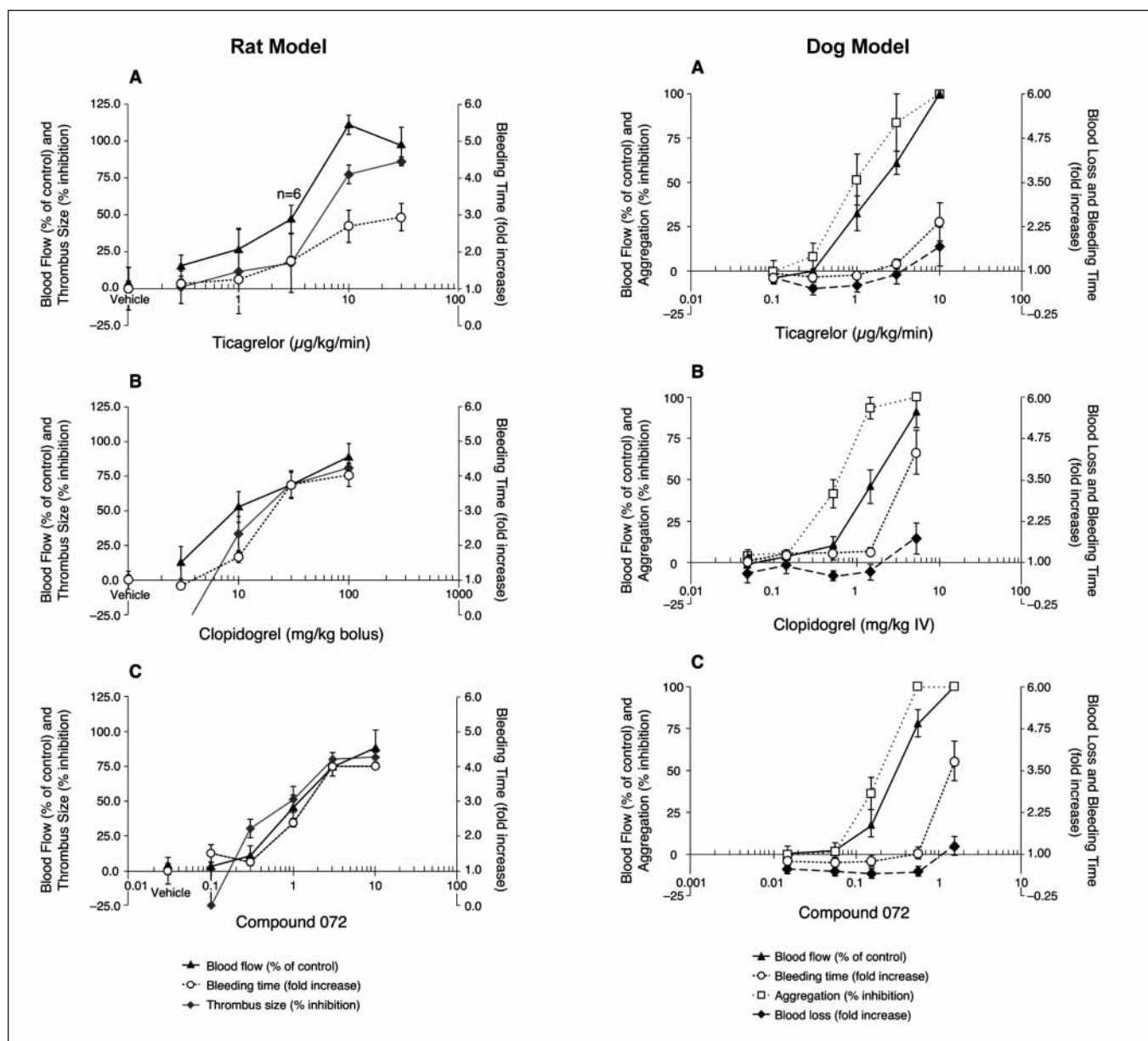
from the ADP binding site (Figs. 3, ► 6) (31, 32, 34, 35). In these studies, ADP acted as a partial agonist of P2Y<sub>12</sub>, whereas 2MeS-ADP inhibited binding of both ticagrelor and ADP. Ticagrelor competitively inhibited 2MeS-ADP-induced receptor signalling and non-competitively inhibited ADP-induced receptor signalling. These findings suggest that ticagrelor does *not* prevent ADP binding but rather inhibits ADP signalling by inactivating the receptor and thus blocking ADP-induced receptor conformational change and G-protein activation; the receptor thus remains functional upon dissociation of the drug molecule.

Reversibility of binding offers more rapid offset of the platelet inhibitory effect, and it may be associated with uncoupling of anti-



**Figure 6: Concentration-response curves for ADP-induced (A) and for 2MeS-ADP-induced (B) P2Y<sub>12</sub> receptor activation as measured by GTP $\gamma$ S binding, run in the absence and in the presence of increasing concentrations of ticagrelor.** Ticagrelor demonstrated different modes of

inhibition for each ligand: for 2MeS-ADP, the curves shifted to the right in a dose-dependent manner; for ADP, the curves both shifted to the right and demonstrated a decrease in  $E_{max}$ . Reproduced with permission from van Giezen et al. (32).



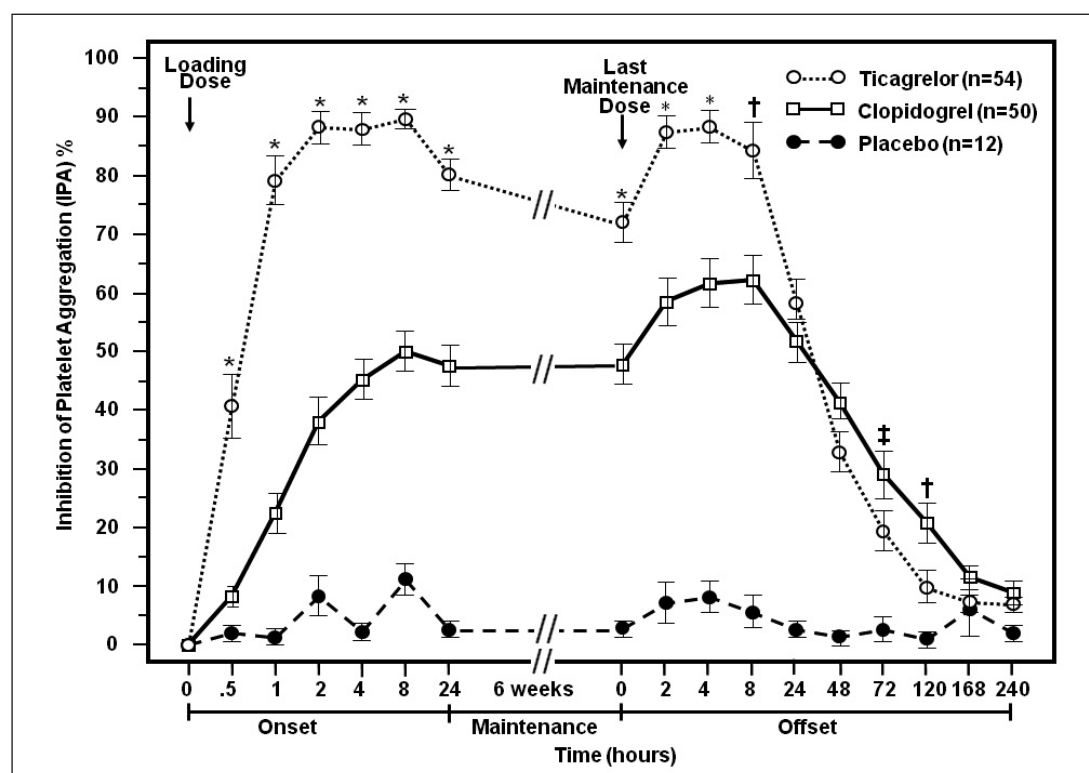
**Figure 7:** Antithrombotic and bleeding effects in rat (left) and dog (right) models of haemostasis and thrombosis according to dose of ticagrelor (A), clopidogrel (B), or AZD11703072 (a compound indistinguishable from prasugrel) (C). In the dog model, the ratio of the doses resulting in a 3.5-fold increase in tongue bleeding time to the doses resulting in 50% restoration of blood flow were >5.2 for ticagrelor, 2.3 for clopidogrel

and 4.3 for the prasugrel surrogate. Comparable results were observed in the rat model, suggesting that the binding characteristics of ticagrelor are associated with a wider separation between antithrombotic effects and bleeding effects than that seen with irreversibly binding thienopyridines. Reproduced with permission from van Giezen et al. (36).

thrombotic and bleeding effects (► Fig. 7) (31, 36). In a canine model of thrombosis and haemostasis, ticagrelor was associated with a greater ratio of the dose resulting in 3.5-fold increase in tongue bleeding time to the dose resulting in 50% restoration of blood flow (>5.2; a 3.5-fold increase in bleeding time was not reached even at the highest dose) than was seen with both clopidogrel (2.3) and a compound indistinguishable from prasugrel (4.3). Similarly, in a rat model, the ratio of the dose resulting in a three-fold increase in tail bleeding time to the dose resulting in 50% res-

toration of blood flow was 9.7 for ticagrelor, versus 2.0 with clopidogrel and 1.4 for a prasugrel surrogate.

In clinical studies, ticagrelor produced greater inhibition of ADP-mediated platelet aggregation than clopidogrel. Peak inhibitory effect correlated with maximum plasma concentration, and the reversibility of effect was illustrated by declining levels of inhibition at 24 hours (h) after the final dose in a 28-day study (28). The findings in the Dose confirmation Study assessing anti-Platelet Effects of AZD6140 vs. clopidogrel in non-ST-segment Elev-



**Figure 8: Inhibition of platelet aggregation (%) (IPA, 20  $\mu$ M adenosine diphosphate, final extent) by protocol time and treatment.** Data expressed as mean  $\pm$  standard error. \* $P < 0.0001$ , † $P < 0.005$ , ‡ $P < 0.05$ , Ticagrelor vs Clopidogrel. Adapted with permission from Gurbel et al. (66).

ation myocardial infarction 2 (DISPERSE-2), a study comparing ticagrelor (90 or 180 mg bid) with clopidogrel (300 mg loading dose followed by 75 mg/d) in patients with non-ST-elevation ACS, are consistent with a separation of therapeutic effect and bleeding risk, as suggested by preclinical studies (37, 38). Ticagrelor, compared to clopidogrel, resulted in a greater inhibition of platelet aggregation, a trend toward a reduced risk of myocardial infarction and a similar incidence of major/minor or major bleeding.

A potential explanation for the above findings is that the distinct mechanism and site of P2Y<sub>12</sub> receptor binding of ticagrelor allow platelet activation in environments characterised by high local concentrations of ADP, thus better preserving an essential haemostatic response as compared to irreversible blockade.

### Cangrelor

Cangrelor, a parenteral ATP analogue resistant to enzymatic degradation, is a reversible P2Y<sub>12</sub> receptor antagonist (Fig. 4) (27, 39, 40). It does not require metabolic activation, exhibits a rapid onset of effect after parenteral administration, and has a very rapid offset of effect following infusion cessation (40–42). As with ticagrelor, preclinical studies suggested a separation between platelet inhibition/antithrombotic effects and bleeding time (43–45). In clinical studies, cangrelor produced greater inhibition of platelet aggregation than did clopidogrel and augmented the degree of inhibition when administered after clopidogrel (indicating binding to unoccupied receptors) (40). In a phase II trial comparing cangrelor (18–24 h infusion at 4  $\mu$ g/kg/min) with abciximab (0.25 mg/kg bolus, followed by 0.125–10  $\mu$ g/min for up to 12 h) in patients

undergoing PCI, maximum platelet inhibition was achieved within 15 min after cangrelor administration, with a return towards baseline thereafter, while inhibition persisted with abciximab (42). Mean inhibition of ADP-induced aggregation was 100% in both groups at completion of the respective infusions, and there were no differences in the rates of either major/minor bleeding or adverse cardiac events, although there was a trend towards greater prolongation of the bleeding time and lower platelet counts with abciximab.

Since cangrelor requires parenteral administration, its use is restricted to hospitalised patients. It has also been demonstrated that simultaneous administration of cangrelor and clopidogrel results in a diminution of antiplatelet response to the latter drug after the cangrelor infusion is stopped (46). This effect may be explained by competition between the active clopidogrel metabolite and cangrelor for the P2Y<sub>12</sub> receptor binding site. A phase III clinical trial comparing cangrelor with clopidogrel was recently halted because no significant differences in measures of clinical effectiveness were observed between the two agents.

### Potential consequences of reversible and irreversible P2Y<sub>12</sub> receptor binding: Focus on bleeding

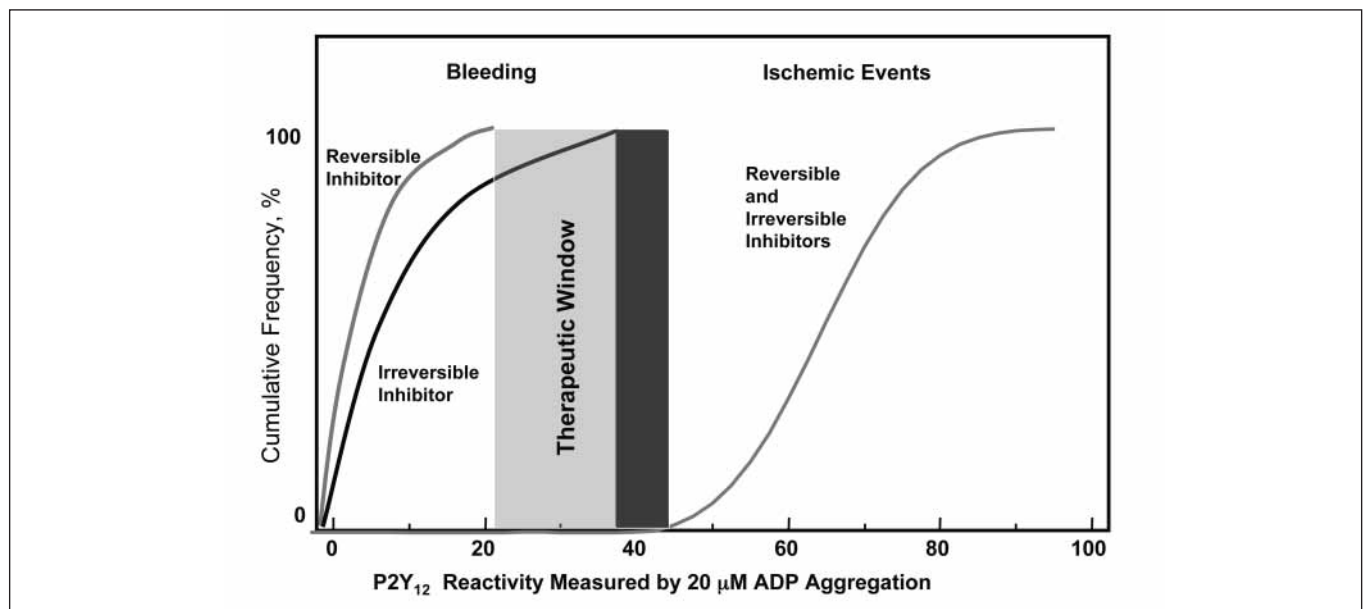
A strong association has been found between major bleeding and mortality in patients with ACS. In a pooled analysis of over 34,000 patients enrolled in the Organization to Assess Strategies for

Ischemic Syndromes (OASIS) Registry, OASIS-2 and the Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) studies, patients who experienced major bleeding had a significantly higher incidence of death within 30 days than patients who did not have major bleeding (12.8% vs 2.5%) (47). In addition, patients with major bleeding had a 4.5-fold greater risk of myocardial infarction and a 6.5-fold greater risk of stroke within 30 days, highlighting the importance of efforts to minimise bleeding in this patient population.

Although dual antiplatelet therapy with clopidogrel and aspirin is a cornerstone of treatment for ACS, the addition of clopidogrel to aspirin is known to increase bleeding risk. Patients undergoing coronary artery bypass surgery (CABG) have been a particular area of focus for several reasons. First, CABG is required during initial hospitalisation in approximately 7–10% of patients with non-ST-segment elevation ACS (48, 49). Second, like ACS, CABG-related bleeding is associated with both short- and long-term cardiovascular events (50–53). Third, the surgical community has devoted substantial effort to preventing bleeding and associated complications. A meta-analysis of 11 prospective and retrospective studies in approximately 4,000 patients undergoing CABG found that surgical blood loss was significantly greater in patients who underwent surgery while taking clopidogrel than in patients not taking clopidogrel, as were transfusion requirements, the rate of surgical re-exploration for bleeding and the overall length of hospital stay (54). Similarly, a structured review of 23 studies including approximately 3,500 patients exposed to clopidogrel within seven days before CABG reported an increased risk for major

bleeding and related complications, including blood product transfusions and re-operation (55). In the CURE study, clopidogrel-treated patients proceeding to CABG during their initial hospitalisation had a 44% reduction in the combined clinical end point of cardiovascular death, myocardial infarction, or ischaemic stroke compared with patients receiving aspirin alone, up to the time of CABG (56). However, patients discontinuing clopidogrel less than five days prior to the surgery also experienced a statistically significant increase in major and life-threatening post-operative bleeding. In response to the available data, current guidelines recommend discontinuation of clopidogrel treatment at least five days before surgery (19–21).

The experience to date with prasugrel heightens concerns for bleeding potential (26, 57). Among patients participating in the TRITON-TIMI 38 trial who underwent CABG, TIMI major bleeding occurred in 13.4% of prasugrel-treated patients versus 3.2% of clopidogrel-treated patients (hazard ratio for prasugrel, 4.73) (26). Given the documented bleeding hazard associated with thienopyridine P2Y<sub>12</sub> receptor antagonists and the suggestion that bleeding increases with the degree of irreversible receptor inhibition, a major unanswered question is whether a similar degree of ADP-mediated inhibition with reversibly binding antagonists such as ticagrelor and cangrelor will offer a safety advantage for patients with ACS. In addition to reversible inhibition, accessibility of the P2Y<sub>12</sub> receptor to ADP, as might be encountered in conditions of sudden haemostatic challenge, may also lessen the risk of bleeding (discussed below). Phase II data for ticagrelor (DISPERSE-2) (37, 38) suggest that potent P2Y<sub>12</sub> blockade with the reversibly binding



**Figure 9:** Cumulative frequency distribution of P2Y<sub>12</sub> reactivity measured by ADP-induced aggregation in patients with bleeding and in patients with ischaemic events. Preclinical data suggest that reversible P2Y<sub>12</sub> blockage may be associated with a wider therapeutic window than that of irreversible inhibition. Recent translational research studies have demonstrated a threshold of platelet reactivity associated with ischaemic

event occurrence. However, the relation of P2Y<sub>12</sub> reactivity to bleeding is less well understood. The optimal goal of antiplatelet therapy would be to place the patient within the therapeutic window, as done with INR in warfarin-treated patients. ADP, adenosine diphosphate. Adapted with permission from Gurbel et al. (67).

agent ticagrelor may provide protection against ischaemic events without significantly increasing bleeding risk.

Several mechanisms may explain the potential benefits of reversibly binding agents with respect to bleeding risk. ADP was the first nucleotide identified within blood that could account for changes in platelet biology upon exposure to a foreign surface. Following mesenteric artery injury in P2Y<sub>12</sub>  $-/-$  mice, platelet adhesion and activation and thrombus growth and stability are reduced. The multiple and diverse effects attributable to P2Y<sub>12</sub> result from its participation at several levels of platelet biology. First, G-protein-coupled signalling up-regulates other surface receptors, including GPIIb/IIIa, GPIIb/IIIa, GPVI and protease-activated receptor (PAR)-1, that are responsible for adhesion, aggregation and activation by collagen and thrombin, respectively. Second, platelet activation causes a release of ADP from dense granules, constituting a positive biofeedback loop with recruitment of platelets and further activation (9, 58). Taken together, available data show that ADP is an important secondary agonist in clot stability, particularly following vascular injury (59).

The importance of ADP-induced platelet aggregation in maintaining haemostatic potential following invasive procedures and even minor trauma is supported by several lines of evidence. ATP, released by exocytosis from damaged erythrocytes, leukocytes and platelets, is converted to ADP by ecto-ATPases. In turn, ADP interacts with P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors to induce platelet aggregation (60). Platelet P2Y<sub>12</sub> receptors are regulated by several distinct mechanisms. One mechanism of P2Y receptor regulation following activation is achieved through desensitisation and trafficking. Specifically, agonist-induced P2Y<sub>1</sub> receptor desensitisation provokes its internalisation in platelets (61). Although P2Y<sub>12</sub> receptors

are rapidly and transiently internalised, most of the receptors remain at the plasma membrane. The differential regulation of P2Y receptors suggests that P2Y<sub>12</sub> in particular may play a vital role in the preservation of haemostatic potential of otherwise unresponsive platelets (61). It has been known for several decades that platelets become refractory to activation by ADP *in vitro* (62). An ability to protect platelets from desensitisation through up-regulation of CD39/ATP diphosphorylase, expressed on the endothelial surface, suggests strongly that the nucleotide is responsible for rapid receptor internalisation (63).

The final mechanism of platelet regulation, and possibly adaptation, that requires consideration is programmed cell death, or apoptosis. While ADP induces P2Y<sub>1</sub> receptor-mediated apoptosis in human astrocytoma cells, the reverse is true of subsequent P2Y<sub>12</sub> activation (64). Indeed, activation of P2Y<sub>12</sub> receptors by either ADP or the synthetic analogues attenuates tumor necrosis factor- $\alpha$ -mediated cellular apoptosis through ERK 1/2, Akt and JNK phosphorylation (64).

Considered collectively, a platelet's ability to bind even a small number of ADP molecules at physiologic states may provide a continuous level of protective haemostatic functionality. Moreover, ADP accessibility to the P2Y<sub>12</sub> receptor could prove particularly important following traumatic vascular injury from invasive procedures or surgery.

The reversible binding characteristics of non-thienopyridine platelet antagonists may allow discontinuation of treatment closer to the time of CABG or other invasive procedures, reducing both the risk of thrombotic events before the operation and the risk of perioperative bleeding. Additional insights and the potential translation of fundamental mechanisms of P2Y<sub>12</sub> receptor inhibition to the risk-benefit profile of an emerging generation of therapies will be derived from the Platelet Inhibition and Patient Outcomes (PLATO) trial, a phase III trial examining the safety and efficacy of ticagrelor compared with that of clopidogrel in approximately 18,000 patients with either non-ST-elevation ACS or ST-elevation ACS (65). In the PLATO trial, treatment with ticagrelor, an agent associated with greater platelet inhibition than clopidogrel, was similarly associated with a lower prevalence of the composite ischaemic endpoint. The primary safety endpoints in PLATO did not differ between groups but non-CABG related major bleeding by PLATO and TIMI criteria were greater in the ticagrelor group. However, the numerically lower CABG bleeding events observed in the ticagrelor group despite the recommendation that the study drug be withheld for 5 days in the clopidogrel group and 24–72 h in the ticagrelor group, appeared to counter-balance the increased non-CABG related major bleeding and drove the primary endpoint of major bleeding to be no different between groups. In the phase II ONSET-OFFSET Study, the slope of the offset of the antiplatelet effect of ticagrelor was shown to be greater for ticagrelor than clopidogrel and may explain the differences in CABG-related bleeding in PLATO between the 2 groups (► Fig. 8) (66). Further prospective studies are clearly required to demonstrate the relation of bleeding to platelet function in patients treated with reversible versus irreversible P2Y<sub>12</sub> inhibitors and at this time, the optimal *ex vivo* measurements to determine safety and efficacy remain uncertain.

### Conflict of interest/disclosure

#### Dr. Paul A. Gurbel

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Finally, it is conceivable that, similar to the “ischaemia threshold” suggested by recent translational studies (8), there may also be a “bleeding threshold” (► Fig. 9) (67). In this context, the goal of antiplatelet therapy would be to place the patient within an optimal therapeutic window, as is routinely done with the international normalised ratio (INR) among warfarin-treated patients. The preclinical data discussed previously suggest that reversible platelet P2Y<sub>12</sub> blockade may be associated with a wider therapeutic window than that of irreversible inhibitors (36).

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