Bleeding manifestations of congenital and drug-induced defects of the platelet $P_2Y_{12}$ receptor for adenosine diphosphate

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Summary
$P_2Y_{12}$, one of the two platelet receptors for adenosine diphosphate (ADP), plays a central role in platelet function. Defects of $P_2Y_{12}$ should be suspected when ADP, even at high concentrations ($\geq 10 \mu M$), is unable to induce full, irreversible platelet aggregation. Patients with congenital $P_2Y_{12}$ defects display a mild-to-moderate bleeding diathesis of variable severity, characterised by mucocutaneous bleeding and excessive post-surgical and post-traumatic blood loss. Drugs that inhibit $P_2Y_{12}$ are potent antithrombotic drugs, attesting the central role played by $P_2Y_{12}$ in platelet thrombus formation. Clopidogrel, the most widely used drug that inhibits $P_2Y_{12}$, is effective both in monotherapy and in combination with acetylsalicylic acid (ASA). Its most important drawback is the inability to inhibit adequately $P_2Y_{12}$-dependent platelet function in about 1/3 of patients, at the recommended therapeutic doses. The incidence of bleeding events is similar in ASA-treated and clopidogrel-treated patients; however, the combination of ASA and clopidogrel causes more bleeding than each drug in monotherapy. Compared to clopidogrel, new drugs inhibiting $P_2Y_{12}$, such as prasugrel and ticagrelor, decrease the risk of cardiovascular events and increase the risk of bleeding complications, because they adequately inhibit $P_2Y_{12}$-dependent platelet function in the vast majority of treated patients.

Keywords
ADP receptors, antiplatelet drugs, coronary syndrome, inherited / acquired platelet disorders, platelet pharmacology

Role of $P_2Y_{12}$ in platelet function

Two distinct receptors for ADP are expressed on platelets: the $G_q$-coupled $P_2Y_1$ and the $G_i$-coupled $P_2Y_{12}$ (Fig. 1). Concomitant activation of both the $G_q$ and $G_i$ pathways by ADP is necessary to elicit normal aggregation (1, 2).

$P_2Y_{12}$ contains 342 amino acid residues, including four extracellular cysteine (Cys) residues at positions 17, 97, 175 and 270: Cys 97 and Cys 175, which are linked by a disulphide bridge, are important for receptor expression (3–5). $P_2Y_{12}$ receptors exist predominantly as homo-oligomers situated in lipid rafts. It has been shown that, upon treatment with the active metabolite of clopidogrel (which inhibits $P_2Y_{12}$ function), the homo-oligomers are disrupted into non-functional dimers and monomers that are sequestered outside the lipid rafts (3).

$P_2Y_12$ is coupled to inhibition of adenyl cyclase activity mostly through activation of $G_{0i2}$ (Fig. 1). It must be noted however that, although inhibition of adenyl cyclase is a key feature of platelet activation by ADP, it bears no causal relationship to platelet aggregation (6). Several studies demonstrated a crucial role for different isoforms of phosphoinositide 3-kinase (PI3-K) in ADP-dependent $P_2Y_{12}$ receptor-mediated platelet activation (Fig. 1) (6). Although ADP by itself is unable to cause significant secretion of platelet dense granules, its interaction with $P_2Y_{12}$ greatly amplifies platelet secretion induced by agonists such as thromboxane A$_2$ (TXA$_2$) (7–9) and thrombin receptor activating peptide (Fig. 1) (10). $P_2Y_{12}$ plays an essential role in the stabilisation of platelet aggregates induced by thrombin (1–13) or TXA$_2$ (14).

Early studies demonstrated that $P_2Y_{12}$ is an important mediator of shear-induced platelet aggregation by using platelets from individuals treated with the anti-thrombotic drug ticlopidine (15) or from a patient with congenital $P_2Y_{12}$ deficiency (16). This effect of $P_2Y_{12}$, which was later confirmed using specific, direct $P_2Y_{12}$ antagonists (6), is dependent upon PI3-K activation (17). Although inhibition of adenyl cyclase via $G_{0i2}$ by ADP bears no causal relationship to platelet activation, it may substantially contribute to platelet thrombus formation in vivo by counteracting the antiplatelet effect of prostacyclin or other substances that stimulate adenyl cyclase (Fig. 1) (18).

$P_2Y_{12}$ shares with $P_2Y_1$ the ability to contribute to collagen-induced platelet microparticle formation in whole blood, and to contribute to the formation of platelet-leukocyte aggregates mediated by platelet surface P-selectin exposure, which results in tissue factor exposure at the surface of leukocytes (19–22). However, only the $P_2Y_{1}$ receptor was found to be involved in the exposure of phosphatidylserine by thrombin or other platelet agonists (21, 23).
Cattaneo: Bleeding in P2Y12 defects

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platelet levels of cyclic AMP or the phosphorylation of vasodilator-stimulated phosphoprotein (VASP) (33) after the exposure of platelets to PGE1, should be used to confirm the diagnosis.

Molecular defects

Severe P2Y12 deficiency

The P2Y12 gene of five of the six patients with severe P2Y12 deficiency who have been described displayed base pair deletions in the open reading frame, resulting in frameshifts and premature truncation of the protein, which were homozygous in two patients (30, 34). Two of the remaining three patients (sisters MG and IG [13]) displayed haploinsufficiency in their remaining allele (35), while the other allele of the fifth patient (28) did not display any mutation, which suggests that he has an additional, as yet unknown mutation that silences his normal allele (36). The remaining patient with severe P2Y12 deficiency is homozygous for a single nucleotide substitution in the transduction initiation codon (ATG to AGG) (30).

Figure 1: Simplified, schematic representation of the effects of the interaction of adenosine diphosphate (ADP) with the platelet P2Y1 and P2Y12 receptors. ATP, adenosine triphosphate; G, G-protein; AC, adenyl cyclase; cAMP, cyclic adenosine diphosphate; VASP: vasodilator-stimulated phosphoprotein; PGE1, prostaglandin E1 (prostacyclin); PKA, protein kinase A.

24) and in tissue factor-induced thrombin formation in platelet-rich plasma (21).

Congenital defects of P2Y12

Congenital P2Y12 deficiency is an autosomal recessive disorder. The first patient with severe P2Y12 deficiency (VR) was described in 1992 (25). He had a lifelong history of excessive bleeding, prolonged bleeding time, reversible aggregation in response to weak agonists and impaired aggregation in response to low concentrations of collagen or thrombin. However, the most typical feature was that ADP, even at very high concentrations (>10 μM), is unable to induce full, irreversible platelet aggregation. In addition, the patient's platelets displayed: i) no inhibition by ADP of prostaglandin E1 (PGE1)-stimulated platelet adenyl cyclase; ii) normal shape change and borderline-normal mobilization of cytoplasmic Ca2+ induced by ADP; iii) presence of approximately 30% of the normal number of binding sites for [33P]2MeSADP on formalin-fixed platelets (which are associated with the ADP receptor P2Y12) (25).

Five additional patients with severe P2Y12 deficiency, belonging to four kindred, and seven patients with dysfunctional P2Y12 or partial P2Y12 deficiency were subsequently described (8, 27–32).

The diagnosis of P2Y12 defects is rather simple: they should be suspected when ADP, even at relatively high concentrations (≥10 μM), is unable to induce full, irreversible platelet aggregation, while inducing normal shape change. Tests that evaluate the degree of inhibition of adenyl cyclase by ADP, by measuring either the

Patients with dysfunctional P2Y12 or with partial P2Y12 deficiency

Analysis of the P2Y12 gene of a patient (AC), who displayed a defect of P2Y12 function but normal [33P]2MeSADP platelet binding sites, revealed, in one allele, a G to A transition changing the codon for Arg256 in TM6 to Gln and, in the other, a C to T transition changing the codon for Arg265 in EL3 to Trp: neither mutation interfered with receptor surface expression but both altered receptor function (27). A heterozygous point mutation in the same region of the molecule, which changed codon 258 coding for proline (CCT) to threonine (ACT) (Pro258Thr), was described in a patient with mild bleeding disorder and severely impaired ADP-induced platelet aggregation (31). Since the proline to threonine substitution alters the protein hydrophobicity, size and rotational mobility, it is likely to affect the function of P2Y12. Finally, a heterozygous mutation, predicting a lysine to glutamate (Lys174Glu) substitution in P2Y12, was identified in one patient with mild type 1 von Willebrand disease (VWD) (32). Platelets from this patient showed reduced and reversible aggregation in response to ADP, up to 10 μM. The reduced response was associated with an approximate 50% reduction in binding of [3H]2MeS-ADP. Considering that Lys174 is situated in the second extracellular loop of P2Y12, adjacent to Cys175, which may be important for the expression of the ADP binding site receptor, and that a haemagglutinin-tagged Lys174Glu P2Y12 variant showed surface expression in Chinese hamster ovary cells, it is likely that the Lys174Glu mutation is responsible for disruption of the ADP binding site of the receptor.

It is interesting to note that, for reasons that are presently unclear, two patients with heterozygous dysfunctional P2Y12 (Pro258Thr and Lys174Glu) display a much more severe impair-
ment of ADP-induced platelet aggregation compared to the two patients who are heterozygous for P2Y12 deficiency (8, 28) and to the two children of patient AC, who are heterozygous for the Arg265Gln mutation (27).

### Bleeding manifestations

Patients with defects of P2Y12 experience mucocutaneous bleeding and excessive post-surgical or post-traumatic blood loss. The severity of their bleeding diathesis is variable. The bleeding scores of patient VR and of the two sisters MG and IG, which was calculated using a standardised questionnaire that was developed to investigate patients with type-1 von Willebrand disease (37), were 8, 7 and 13, respectively, (normal values ≤ 3) (unpublished data). The degree of prolongation of their bleeding times was also variable, reflecting the severity of their clinical bleeding scores: 15 and 20 minutes (min) (results of two measurements in patient VR), 20 min (patient MG) and >30 min (patient IG) (normal values <8 min). After extensive investigation of haemostasis parameters, which included measurement of the activity of clotting and fibrinolytic factors and the search for known polymorphisms of haemostasis proteins, we found no explanation for the discrepancy in the severity of bleeding manifestations in the two sisters MG and IG.

The bleeding score of a patient with heterozygous P2Y12 deficiency (GL, the son of patient MG) was normal; however, it must be noted that this young boy had not yet experienced situations that could challenge the haemostatic system at the time of our investigation. His bleeding time, despite the mild defect of P2Y12, was prolonged (13 min).

### Drugs inhibiting P2Y12

#### “Old” drugs

Ticlopidine and clopidogrel, which belong to the family of thienopyridines, are pro-drugs that need to be converted \textit{in vivo} by the hepatic cytochrome P-450 (CYP) enzymatic pathway to active metabolites, which covalently bind to P2Y12 by forming a disulfide bond with cysteine residues, thereby irreversibly inhibiting the receptor (39). Recently, paraoxonase-1 was also shown to play a critical role in clopidogrel bioactivation (40). Several randomised clinical trials documented the clinical efficacy of these drugs in the prevention of major adverse cardiovascular events (MACE) (41). Their efficacy was essentially similar to that of acetylsalicylic acid (ASA) in patients with stable disease of cerebral or coronary arteries (42–44). For patients with acute coronary syndromes, undergoing medical treatment only or in combination with percutaneous coronary intervention (PCI), the addition of thienopyridines to acetylsalicylic acid (ASA) proved highly efficacious. In

The intravenous infusion of the vasopressin analogue desmopressin (0.3 μg/kg) shortened the prolonged bleeding time of patient VR from 20 min to 8.5 min (15). After the infusion of desmopressin, which was repeated twice at 24-hour (h) intervals, the patient underwent a surgical intervention for disc hernia repair, which was not complicated by excessive bleeding. Although the efficacy of desmopressin in reducing bleeding complications of patients with defects of primary haemostasis is anecdotal (38), its administration is generally without serious side effects.

### Table 1: Characteristics of the patients with congenital P2Y12 deficiency.

<table>
<thead>
<tr>
<th>Patient identification [reference]</th>
<th>P2Y12 mutations</th>
<th>Platelet aggregation induced by ADP ≥10 μM</th>
<th>History of abnormal bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>VR [25, 34]</td>
<td>p.[Gln98fs]+[Gln98fs]</td>
<td>Reduced and reversible</td>
<td>Yes</td>
</tr>
<tr>
<td>ML [28, 36]</td>
<td>p.[Phe240fs]+[?]*</td>
<td>Reduced and reversible</td>
<td>Yes</td>
</tr>
<tr>
<td>IG [8, 35]</td>
<td>p.[0]+p.Thr126fs#</td>
<td>Reduced and reversible</td>
<td>Yes</td>
</tr>
<tr>
<td>MG [8, 35]</td>
<td>p.[0]+p.Thr126fs#</td>
<td>Reduced and reversible</td>
<td>Yes</td>
</tr>
<tr>
<td>OSP-1 [29]</td>
<td>p.[0]+[0]§</td>
<td>Reduced and reversible</td>
<td>Yes</td>
</tr>
<tr>
<td>?? [30]</td>
<td>p.[Gly12fs]+[Gly12fs]</td>
<td>Reduced and reversible</td>
<td>Yes</td>
</tr>
<tr>
<td>CL [28, 36]</td>
<td>p.[Phe240fs]+[=]</td>
<td>Full and irreversible</td>
<td>No</td>
</tr>
<tr>
<td>GL [8, 35]</td>
<td>p.[0]+[=]§</td>
<td>Full and irreversible</td>
<td>No</td>
</tr>
<tr>
<td>AC [27]</td>
<td>p.[Arg256Gln]+[Arg265Trp]</td>
<td>Reduced and reversible</td>
<td>Yes</td>
</tr>
<tr>
<td>MC [27]</td>
<td>p.[Arg265Trp]+[=]</td>
<td>Full and irreversible</td>
<td>No</td>
</tr>
<tr>
<td>FC [27]</td>
<td>p.[Arg265Trp]+[=]</td>
<td>Full and irreversible</td>
<td>No</td>
</tr>
<tr>
<td>GS [31]</td>
<td>p.[Pro258Trp]+[=]</td>
<td>Reduced and reversible</td>
<td>Yes</td>
</tr>
<tr>
<td>PII.1 [32]</td>
<td>p.[Lys174Glu]+[=]</td>
<td>Reduced and reversible</td>
<td>Yes*</td>
</tr>
</tbody>
</table>

CL: daughter of ML; GL: son of MG, who is the sister of IG. § Failure of expression of the P2Y12 protein (p.[0]) in patient OSP-1 was associated with homozygous single nucleotide substitution in the transduction initiation codon (ATG to AGG). # p[0] was associated with partial or complete P2Y12 gene deletion in patients IG, MG and GL.
contrast, the combination of clopidogrel and ASA was not more effective than ASA in monotherapy in low-moderate risk patients with stable disease, but increased the incidence of bleeding (see later).

Despite its proven antithrombotic efficacy, clopidogrel has some important drawbacks (44): i) its antiplatelet effects are delayed, due to the need for metabolism of the pro-drug; ii) there is substantial inter-individual variability in platelet inhibition, with about 1/3 of treated patients who display very low or no response to the recommended therapeutic doses; iii) its ability to irreversibly inhibit P2Y12 may represent a problem for patients who need to undergo coronary bypass (CABG) surgery, because the incidence of post-operative bleeding complications is higher in patients not treated with clopidogrel. While the onset of action of clopidogrel can be accelerated by giving patients a loading dose of 300–600 mg, the solution of the other two problems appears more problematic (44).

The high inter-individual variability of the response to clopidogrel, which is associated with genetic abnormalities of CYP and with negative interference of environmental factors (including common adjunctive medications, such as proton pump inhibitors) is a clinically relevant issue, as it has been demonstrated that poor responders are not adequately protected from MACE. Tailored treatment of patients, based on the results of platelet function tests or of CYP genotyping, has been proposed to solve the problem of clopidogrel resistance (44). This approach cannot be recommended in daily clinical practice yet, because the best laboratory method to monitor the effects of clopidogrel on platelet function still needs to be identified, standardised (for pre-analytical and analytical variables) and validated in the clinical setting. Several recent studies demonstrated that the agreement among different laboratory tests to identify poor responders is rather low and that assessment of platelet response to clopidogrel is highly test-specific (45–50) and that the search for loss of function mutations of CYP is not very accurate in predicting the response to clopidogrel (51–53). Mostly based on the aforementioned consideration, a recent consensus paper concluded that until the results of large-scale trials of personalised antithrombotic treatment are available, the routine use of platelet function measurements in the care of patients with cardiovascular disease cannot be recommended (54). The results of one of these trials, GRAVITAS, which were presented at the Congress of the American Heart Association in November 2010, showed that repeating the loading dose and doubling the maintenance dose of clopidogrel (150 mg daily) in patients who did not display a sufficient degree of inhibition of platelet function (measured with the VerifyNow test) after a first loading dose of the drug and the PCI procedure, did not decrease the incidence of MACE (nor did it increase the incidence of bleeding events), compared to the recommended dose of clopidogrel (75 mg daily). The results of the GRAVITAS trial emphasise the concept that, in the absence of a validated protocol, tailored treatment with clopidogrel based on platelet function testing should not implemented in the clinical practice yet. Therefore, the use of new P2Y12 antagonists that are able to induce predictable and adequate inhibition of platelet function in all patients is desirable.

“New” drugs

Prasugrel is a new thienopyridine, with much more rapid and consistent inhibitory effects on platelet aggregation than clopidogrel. It has a distinct chemical structure, which permits conversion to its active metabolite with less dependence on CYP enzymes than clopidogrel (44). Consequences of the different metabolism of prasugrel, compared to that of clopidogrel, are (44): 1) faster appearance and higher concentration of its active metabolite in circulating blood; 2) faster and greater mean inhibition of P2Y12-dependent platelet function; 3) no influence of the CYP genotype on its pharmacokinetics, pharmacodynamics and antithrombotic activity; 4) much lower inter-individual variability in inhibition of P2Y12-dependent platelet responses. The aforementioned more favourable characteristics of prasugrel compared to clopidogrel result in greater clinical benefit, as shown by the results of TRITON TIMI-38, which evaluated 13,608 high-risk patients with acute coronary syndromes who required PCI, despite the fact that the incidence of bleeding complications was higher in prasugrel-treated patients compared to clopidogrel-treated patients (see later) (55).

Ticagrelor is a direct P2Y12 inhibitor, which belongs to the new chemical class cyclopentyl-triazolo-pyrimidines: it does not require conversion to an active metabolite and has a half-life of about 7–8.5 h (56, 57). After oral administration, it rapidly and reversibly inhibits P2Y12 via a mechanism that is non-competitive with ADP, suggesting the existence of an independent receptor binding site (58). In phase II trials, ticagrelor more rapidly and effectively inhibited platelet aggregation and with less variability than clopidogrel (59, 60). A study that compared the onset and offset of action of clopidogrel and ticagrelor showed that, despite the greater mean antiplatelet effect of ticagrelor, inhibition of platelet aggregation at 24 h after the last dose was equivalent in ticagrelor- and clopidogrel-treated patients, which is indicative of a faster offset of effect (61). The results of the PLATO trial, in which ticagrelor (180 mg LD, 90 mg b.i.d, MD) was compared to clopidogrel (300–600 mg LD, 75 mg daily MD) for prevention of MACE in patients with non-ST or ST elevation acute coronary syndromes (2/3 of them underwent PCI) showed that, compared to ticagrelor, ticagrelor decreased the incidence of MACE and, very importantly, that of cardiovascular and total mortality, but was associated with higher incidence of spontaneous major bleeding events (see later) (62).

Cangrelor belongs to a family of analogues of ATP that are relatively resistant to breakdown by ectonucleotidases and display high affinity for the P2Y12 receptor, which is reversibly inhibited by the drug (44). Cangrelor does not require conversion to an active metabolite and is immediately active after intravenous infusion, with a half-life of 3–6 min. Two trials, which compared cangrelor to clopidogrel in patients requiring PCI, were prematurely terminated due to insufficient evidence of superiority of cangrelor (63, 64).

Elinogrel, a P2Y12 inhibitor that is active both orally and intravenously, is currently under evaluation in phase III trials (44).
Bleeding events associated with treatment with P2Y12 inhibitors

As already mentioned, patients with congenital P2Y12 defects have a bleeding diathesis of variable severity: it was therefore not unexpected that drugs targeting P2Y12 increase the incidence of bleeding. Although the value of laboratory tests of haemostasis for the prediction of bleeding events in patients with acute coronary syndromes under antithrombotic treatment is extensively being evaluated, a recent study showed that a simple bleeding score, based on six readily available clinical and laboratory variables (female sex, advanced age, elevated serum creatinine and white blood cell count, anaemia, type of acute coronary syndrome), plus the anticoagulation regimen used, may provide a rapid tool to predict the rate of major bleeding in these patients (65).

Major bleeding and the risk of mortality

Severe bleeding complications during antithrombotic therapy have negative consequences, not only because they may be fatal, disabling, and expose the patients to the risks that are associated with blood transfusion: they are also associated with poor prognosis of the patients, whose risk of death is increased during a follow-up of up to one year (66, 67). The nature of the relationship between major bleeding complications and long-term mortality is unclear. Despite the fact that this association remained statistically significant after adjustment for confounders, it is still possible that bleeding may simply be a marker of an underlying severe disease state, which exposes the patient to increased risk of mortality. A direct causal effect of major bleeding on the long-term risk of death is unlikely and, as a matter of fact, it is ruled out by the observation that major bleeding after CABG surgery is not associated with increased mortality (65, 67). Yet, the possibility that non-CABG-related major bleeding may be indirectly causally associated with increased mortality of patients under treatment with antithrombotic drugs is biologically plausible. Antithrombotic drugs are usually withheld in patients who experience major bleeding, and this exposes them to high risk of MACE. In keeping with this hypothesis is the observation that major bleeding was also associated with increased risk of ischaemic events, such as myocardial infarction and stroke (68).

“Old” drugs vs. ASA

Studies that compared old thienopyridines (ticlopidine or clopidogrel) to ASA showed that the risk of bleeding was not significantly different between the two treatment arms (41). This observation is somewhat surprising, considering that in vitro and in vivo experiments demonstrated that ADP plays a more important role in platelet thrombus formation than thromboxane A2. Although there are many plausible explanations for these unexpected results, the high prevalence of non-responders to old thienopyridines, who are not exposed to the risk of bleeding, is the most plausible.

Combined treatment with clopidogrel and ASA

Combined treatment with clopidogrel and ASA is associated with increased bleeding compared to ASA in monotherapy. If this is a fair price to pay when treating patients with ACS, in consideration of the net clinical benefit associated with combined therapy, it is unacceptable for secondary prophylaxis of patients with stable disease or for primary prophylaxis of patients at risk, because the higher bleeding risk is not counterbalanced by antithrombotic efficacy in these settings (41). An unacceptably high incidence of

Table 2: Safety and efficacy of prasugrel, ticagrelor and high-dose clopidogrel, compared to standard dose clopidogrel.

<table>
<thead>
<tr>
<th>RCT [reference]</th>
<th>Patients</th>
<th>Treatments*</th>
<th>Primary end-points</th>
<th>Follow-up</th>
<th>Efficacy HR (95%CI)</th>
<th>Non CABG TIMI major bleeding HR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRITON TIMI38 [55]</td>
<td>ACS with scheduled PCI</td>
<td>1. Prasugrel + ASA 2. Clopidogrel low dose + ASA</td>
<td>CV death, nonfatal stroke, or nonfatal AMI</td>
<td>6–15 months</td>
<td>0.81 (0.73–0.89)</td>
<td>1.32 (1.03–1.68)</td>
</tr>
<tr>
<td>CURRENT OASIS 7 (PCI) [73]</td>
<td>ACS with scheduled PCI</td>
<td>1. Clopidogrel high dose + ASA 2. Clopidogrel low dose + ASA</td>
<td>CV death, AMI, or stroke</td>
<td>30 days</td>
<td>0.86 (0.74–0.99)</td>
<td>1.36 (0.97–1.90)</td>
</tr>
<tr>
<td>CURRENT OASIS 7 [72]</td>
<td>ACS with or without ST elevation</td>
<td>1. Clopidogrel high dose + ASA 2. Clopidogrel low dose + ASA</td>
<td>CV death, AMI, or stroke</td>
<td>30 days</td>
<td>0.94 (0.83–1.06)</td>
<td>1.26 (1.03–1.54)</td>
</tr>
<tr>
<td>PLATO [62]</td>
<td>ACS with or without ST elevation</td>
<td>1. Ticagrelor + ASA 2. Clopidogrel low dose + ASA</td>
<td>CV death**, AMI, or stroke</td>
<td>12 months</td>
<td>0.84 (0.77–0.92)</td>
<td>1.25 (1.03–1.53)</td>
</tr>
</tbody>
</table>

* Low dose clopidogrel: 300 mg loading dose + 75 mg daily, maintenance dose (in the PLATO trial, investigators were allowed to use either 300 mg or 600 mg loading dose clopidogrel). High dose clopidogrel: 600 mg lading dose + 75 mg daily maintenance dose for 1 week + 75 mg maintenance dose for the remaining time of treatment. Doses of ASA were: 75–162 mg daily (ref. 55); 75–325 mg daily (ref. 62); 75/100 mg or 300/325 mg daily (ref. 72 and 73). ** The incidence of cardiovascular death and of death from any cause was significantly decreased by ticagrelor, compared to clopidogrel.
major bleeding complications, not counterbalanced by higher antithrombotic efficacy, characterised also the combined treatment of ASA plus clopidogrel, compared to clopidogrel in monotherapy, in patients at risk of cerebrovascular events (69).

“New” P2Y₁₂ inhibitors vs. clopidogrel

As previously mentioned, the incidence of TIMI-major bleeding complications in prasugrel-treated patients was higher than that in clopidogrel-treated patients who were enrolled in the TRITON TIMI-38 trial. Based on these results, prasugrel is generally considered a more potent antiplatelet agent than clopidogrel, to be used only in high-risk patients or for a short period, while treatment with clopidogrel should be preferred in the remaining situations. However, it is incorrect to say that prasugrel is more potent than clopidogrel, as both ex vivo and in vitro studies demonstrated that the active metabolites of the two compounds have the same potency (70, 71). The different clinical efficacy and safety of prasugrel compared to clopidogrel is mostly explained by the fact that very few treated patients respond poorly to prasugrel (44). Because protection from thrombotic events and exposure to bleeding risk are a function of the degree of inhibition of P2Y₁₂-dependent platelet function, the higher efficacy and the lower safety of prasugrel compared to clopidogrel are simply explained by the fact that prasugrel protects from MACE and exposes to the risk of bleeding more patients than clopidogrel. Based on the results of published studies, it can be predicted that, if tailored treatment with clopidogrel were successful in all patients displaying hyporesponsiveness to the drug, incidences of MACE and bleeding in patients given tailored clopidogrel treatment would be similar to those observed in patients given prasugrel (44).

The observation that ticagrelor, which, like prasugrel, adequately inhibits P2Y₁₂-dependent platelet function in the great majority of treated patients, caused more non-CABG-related TIMI-major bleedings than clopidogrel in the PLATO trial (62), is compatible with the aforementioned considerations. In addition, the incidences of non-CABG-related TIMI-major bleeding events were higher in patients with ACS who were randomised to high-dose clopidogrel (600 mg loading dose plus 150 mg maintenance dose for one week), compared to those randomised to standard dose clopidogrel (300 mg loading dose plus 75 mg maintenance dose), who were enrolled in the CURRENT OASIS-7 trial (72, 73). Despite the different designs of the studies, it is notable that the hazard ratios for TIMI-major bleeding compared to standard dose clopidogrel were similar for high-dose clopidogrel (72, 73), prasugrel (55) and ticagrelor (62).

P2Y₁₂ inhibitors and CABG-related bleeding

Less than 10% of patients with ACS need to undergo CABG surgery. It has been demonstrated that clopidogrel treatment within about four days of the procedure is associated with increased blood loss, reoperation for bleeding, increased transfusion requirements and prolonged intensive care unit and hospital stays (44). For this reason, when the clinical conditions of the patients allow it, clopidogrel is usually withheld for five days before CABG, in order to restore the haemostatic competency of the patient. This is accomplished by the progressive increase in the number of circulating newly formed, non-inhibited platelets, which, after five days of withdrawal of the drug, should represent about 60–70% of the total circulating platelet population: certainly enough to secure normal haemostasis. This procedure was followed for all patients undergoing CABG in randomised clinical trials that compared the new P2Y₁₂ antagonists to clopidogrel. For this reason, the incidence of CABG-related bleeding complications should not be considered when evaluating the risk of bleeding associated with new anti-P2Y₁₂ drugs, for the simple reason that patients were off-treatment when they underwent CABG. Differences among P2Y₁₂ antagonists in this setting should be evaluated on the basis of the time needed to withhold treatment before surgery to restore haemostatic competency. Considering that withholding antiplatelet treatment exposes patients to high risk of MACE, it is obvious that drugs with reversible mechanism of action and short half-life, such as ticagrelor, may be preferable to drugs that irreversibly inhibit the receptor.

The TRITON TIMI-38 trial showed that the incidence of CABG-related major bleeding complications in prasugrel-treated patients was significantly higher than that observed in clopidogrel-treated patients. Based on this observation, the guidelines of the European Society of Cardiology recommend that prasugrel should be stopped for seven days, i.e. longer than the recommended five days for clopidogrel, before CABG surgery (73). However, it is very likely that the higher incidence of bleeding complications among prasugrel-treated patients compared to clopidogrel-treated patients observed in the TRITON TIMI-38 trial occurred in the patients whose CABG surgery had to be performed before the 5th day since drug withdrawal, when the antiplatelet effects of the drugs had not disappeared yet. As a matter of fact, since there is no reason to believe that the rate of production of newly formed platelets is different in prasugrel-treated patients, compared to clopidogrel-treated patients, it should be assumed that after five days of drug withdrawal, 60–70% of uninhibited platelets (enough to secure normal haemostasis) will be circulating in the blood of all patients, independently of their initial treatment. Based on this consideration, I believe that there is no good reason to postpone CABG surgery beyond the 5th day since drug withdrawal in prasugrel-treated patients, because this practice would unjustifiably expose them to the risk of major cardiovascular events for additional days.

Conclusion

The platelet P2Y₁₂ receptor for ADP plays an important role in primary haemostasis, as demonstrated by the observation that both congenital and drug-induced abnormalities of the receptor are associated with abnormal platelet function and increased risk of bleeding. There is a high inter-individual variability in the incidence and severity of bleeding manifestations that are associated with both congenital and drug-induced impairment of P2Y₁₂ function, suggesting that other factors contribute to the risk of
bleeding. The results of randomised clinical trials suggest that the incidence of bleeding complications associated with the use of antiplatelet drugs inhibiting P2Y12 does not vary with the type of drug used, but, rather, with the degree of inhibition of P2Y12 function. The higher incidence of bleeding complications (as well as the lower incidence of MACE) observed in patients treated with the new P2Y12 inhibitors prasugrel and ticagrelor, compared to clopidogrel, is most likely explained by the fact that the new drugs effectively inhibit platelet function in virtually all treated patients, while clopidogrel, at the recommended doses, is inactive in about 1/3 of patients.

Conflict of interest
M. Cattaneo has received honoraria from AstraZeneca, Eli Lilly and Daiichi Sankyo.

References
Cattaneo: Bleeding in P2Y12 defects