Antiplatelet therapies for the treatment of cardiovascular disease

Alan D. Michelson

Abstract | Antiplatelet therapy has been successful in reducing mortality and morbidity in acute myocardial infarction. Recent advances in understanding the molecular basis of the role of platelets in cardiovascular thrombosis have enabled the development of new agents with the potential to further reduce mortality and morbidity. This article reviews the role of platelets in haemostasis and cardiovascular thrombosis, and discusses the benefits and limitations of current and investigational antiplatelet agents in the treatment of cardiovascular disease.

Acute myocardial infarction
Popularly known as a heart attack, this is the death of heart tissue from lack of oxygen, which is usually caused by a clot (thrombosis) in an artery that supplies blood to the heart.

Thrombosis
The development of a blood clot in the circulatory system. Depending on the location of the clot, the resultant loss of circulation can lead to a heart attack (coronary thrombosis) or a stroke (cerebral thrombosis).

Haemostasis
The normal process of stopping bleeding.

Center for Platelet Research Studies, Children's Hospital Boston, Karp 08213, 300 Longwood Avenue, Boston, Massachusetts 02115-5737, USA.
e-mail: alan.michelson@childrens.harvard.edu
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The most common cause of death in the developed world is acute myocardial infarction, which is caused by coronary artery thrombosis. Platelets have a central role in cardiovascular thrombosis. They adhere to the subendothelial matrix after endothelial damage due to a ruptured atherosclerotic plaque, then aggregate with each other to form a prothrombotic surface that promotes clot formation and subsequently vascular occlusion. As a result, therapies targeting key pathways of platelet activation — including thromboxane A_2 synthesis, ADP-mediated signalling and integrin αIIbβ3 (also know as GPIIb–IIIa) signalling — have established a role in the treatment of cardiovascular arterial disease.

The most common of these antiplatelet agents include aspirin, clopidogrel and integrin αIIbβ3 antagonists. The most clear-cut evidence that acute myocardial infarction is a platelet-related disease is the ability of antiplatelet therapy to reduce mortality and morbidity in this clinical setting[1]. However, there remains a substantial incidence of arterial thrombosis in patients on currently available antiplatelet therapy. Limitations of current therapies include weak inhibition of platelet function (for example, by aspirin), blockade of only one pathway of ADP-mediated signalling (for example, by clopidogrel), slow onset of action (for example, of clopidogrel), interpatient response variability with poor inhibition of platelet response in some patients (for example, to clopidogrel), the inability to transform the success of intravenous integrin αIIbβ3 antagonist therapy into oral therapy and the inability to completely separate a reduction in thrombotic events from an increase in bleeding events.

Current antiplatelet therapy can therefore be improved upon, and there have been several promising advances in recent years including: the development of ADP receptor antagonists with a more rapid onset of action, a more potent antiplatelet effect and less patient hyporesponsiveness; targeting new epitopes to try to improve oral integrin αIIbβ3 antagonists; and the development of agents directed against new platelet surface targets such as proteinase-activated receptor 1 (PAR1), platelet glycoprotein VI (GPVI), integrin α2β1, 5-hydroxytryptamine receptor 2A ($5HT_{2A}$), and prostaglandin E2 receptor EP_3 subtype (EP_3) with the goal of better separating reduced thrombotic events from increased bleeding events.

This article provides an overview of the actions and roles of platelets, highlighting the central importance of platelets in cardiovascular disease. Approved antiplatelet drugs, agents that are currently in clinical trials and promising possible future approaches will be discussed.

Roles and actions of platelets

Platelets have a crucial role in haemostasis and thrombosis[2], but they also have important roles in wound healing[3], inflammation[4], antimicrobial host defence[5], angiogenesis[6], and tumour growth and metastasis[7]. Platelets are small subcellular fragments (2–5 μm diameter, ~0.5 μm thickness and 6–10 fl volume) that circulate in the blood for 7–10 days at a concentration of 150–400 x10^9 per litre. Structural features of platelets include granules (dense, lysosomal and α-granules), mitochondria, a cytoskeleton, a surface-connected canalicular system and a dense tubular system, but no nucleus[8]. Platelets are derived from the cytoplasm of megakaryocytes — the only polyploid haematopoietic cells[9]. Polyploid megakaryocytes and their platelet progeny are found only in mammals[10]. In all other animal species, cells involved in haemostasis and blood coagulation are nucleated. The evolutionary events that produced mammalian megakaryocytes and platelets, as well as the biological advantage of this system, remain elusive[10].

Platelet receptors, as the contacts between platelets and their environment, determine the reactivity of platelets with a wide range of agonists and adhesive proteins[11] (FIG. 1). The small size and discoid shape of platelets result in their being pushed to the vessel edge by larger cellular elements (erythrocytes and leukocytes) and by blood flow. This positions platelets near the apical surface of the endothelium, which is optimal for rapidly detecting and responding to vascular damage[12]. When platelets encounter surface-bound activating factors such as von Willebrand factor (VWF) bound to collagen and/or soluble factors released into the blood, they avidly react, bind, spread, secrete and interact with one another and with fibrin to form a plug that seals the damaged surface. Plug formation requires platelets to undergo rapid morphological changes from their resting discoid forms into their active shapes[12]. Spreading allows platelets to flatten over the damaged surface, and the elaboration of long filopods facilitates the recruitment of additional platelets into the wound site. The endothelium, in addition to being a passive barrier between platelets and subendothelial collagen, negatively regulates excessive platelet activation by three active mechanisms involving nitric oxide, the eicosanoid prostacyclin, and the ecto-nucleotidase CD39 (REF. 13) (FIG. 1).

Platelets and cardiovascular disease

Platelets preserve vascular integrity and thereby prevent haemorrhage after injury. However, vascular damage, such as rupture of an atherosclerotic plaque, results in a platelet-dependent thrombus, which may lead to vascular occlusion with resultant hypoxia and infarction of distal tissues. Thrombotic occlusion of a coronary artery results in acute myocardial infarction and thrombotic occlusion of a cerebral artery results in acute ischaemic stroke.

In the venous system, low flow rates and stasis permit the accumulation of activated coagulation factors and the local generation of thrombin, largely without the involvement of platelets[14]. Although venous thrombi contain platelets, the dominant cellular components are trapped erythrocytes. In the arterial circulation, higher flow rates limit fibrin formation by washing out soluble clotting factors[14]. Haemostasis in the arterial circulation requires platelets — to accelerate thrombin formation, to form a physical barrier and to provide a base upon which fibrin can accumulate. Haemostatic plugs and thrombi that form in the arterial circulation are therefore enriched in platelets as well as fibrin, giving them a different appearance from those formed in the venous circulation[14]. When viewed from the perspective of biological evolution, human platelets are part of a beneficial system that reduces the risk of death following trauma or childbirth. The drawback is the risk of unwanted platelet activation, particularly at sites of atherosclerotic disease in the coronary and cerebrovascular circulation[14].

Because of the central role of platelets in arterial thrombosis, antiplatelet therapy has proved beneficial in this setting, as discussed below.

Current and investigational antiplatelet therapies target key pathways of platelet activation (FIG. 1). These targets include platelet surface receptors (for example, P2Y purinoceptor 12 ($P2Y_{12}$), integrin αIIbβ3, integrin α2β1, PAR1, GPVI, glycoprotein 1b, P-selectin, EP3, the $5HT_{2A}$ receptor and the thromboxane prostanoid receptor), signalling molecules (for example, cyclooxygenase 1 (COX1; also known as prostaglandin G–H synthase 1), phosphodiesterases and the β isoform of phosphoinositide 3-kinase (PI3K)) and endothelial products (namely, nitric oxide).

Aspirin

For over 50 years, aspirin (TABLE 1; Supplementary information S1 (figure)) has been the foundation of antiplatelet therapy, and it remains so today. It irreversibly acetylates Ser529 of COX1, rendering the catalytic site of COX1 inaccessible to arachidonic acid and therefore inhibiting the generation of prostaglandin H_2 and, subsequently, thromboxane A_2 (REFS 15,16) (FIG. 1). Inhibition of the release of thromboxane A_2 from platelets blocks platelet activation through the thromboxane receptor (FIG. 1). Oral aspirin is rapidly absorbed from the stomach and small intestine, reaching peak plasma levels in 30–40 minutes and inhibiting platelet function by 60 minutes[17]. However, enteric coated aspirin tablets can take up to 3–4 hours to reach peak plasma levels[17]. The plasma half-life of aspirin is only 15–20 minutes, but the platelet inhibitory effect lasts for the lifespan of the platelets because of the irreversible inactivation of COX1 (REFS 15,17).

In high-risk patients, aspirin reduces vascular death by ~15% and non-fatal vascular events by ~30% — as evidenced by meta-analysis of over 100 randomized trials[1,17]. Aspirin may also be of benefit in the primary prevention of cardiovascular events, but the effect is more modest[1,17]. The very low cost of the drug is a major advantage. Several issues related to the clinical efficacy of aspirin have been reviewed[17] — namely, the optimal dose to maximize efficacy and minimize toxicity, the suggestion that part of the antithrombotic effect of aspirin is unrelated to inhibition of thromboxane A_2 and the possibility that some patients may be aspirin 'resistant' (hyporesponsive). With regard to the first issue, the saturability of the antiplatelet effect of aspirin at low doses, the lack of a dose–response relationship in clinical studies evaluating its antithrombotic effects and the dose-dependence of its side effects support the use of a low dose of aspirin (50–100 mg per day for long-term treatment)[17]. This has been found to be effective in the treatment of various thromboembolic disorders[17]. With regard to the second issue, aspirin has been reported to have inhibitory effects on haemostasis that are COX1-independent, including inhibition of platelet function, enhancement of fibrinolysis and suppression of coagulation[17,18]. However, these effects are dose dependent and therefore inconsistent with the lack of dose dependence of aspirin in randomized clinical trials[1,17]. With regard to the third issue, the concept has arisen that some patients are more resistant to aspirin therapy than other patients[19]. Two recent meta-analyses of published

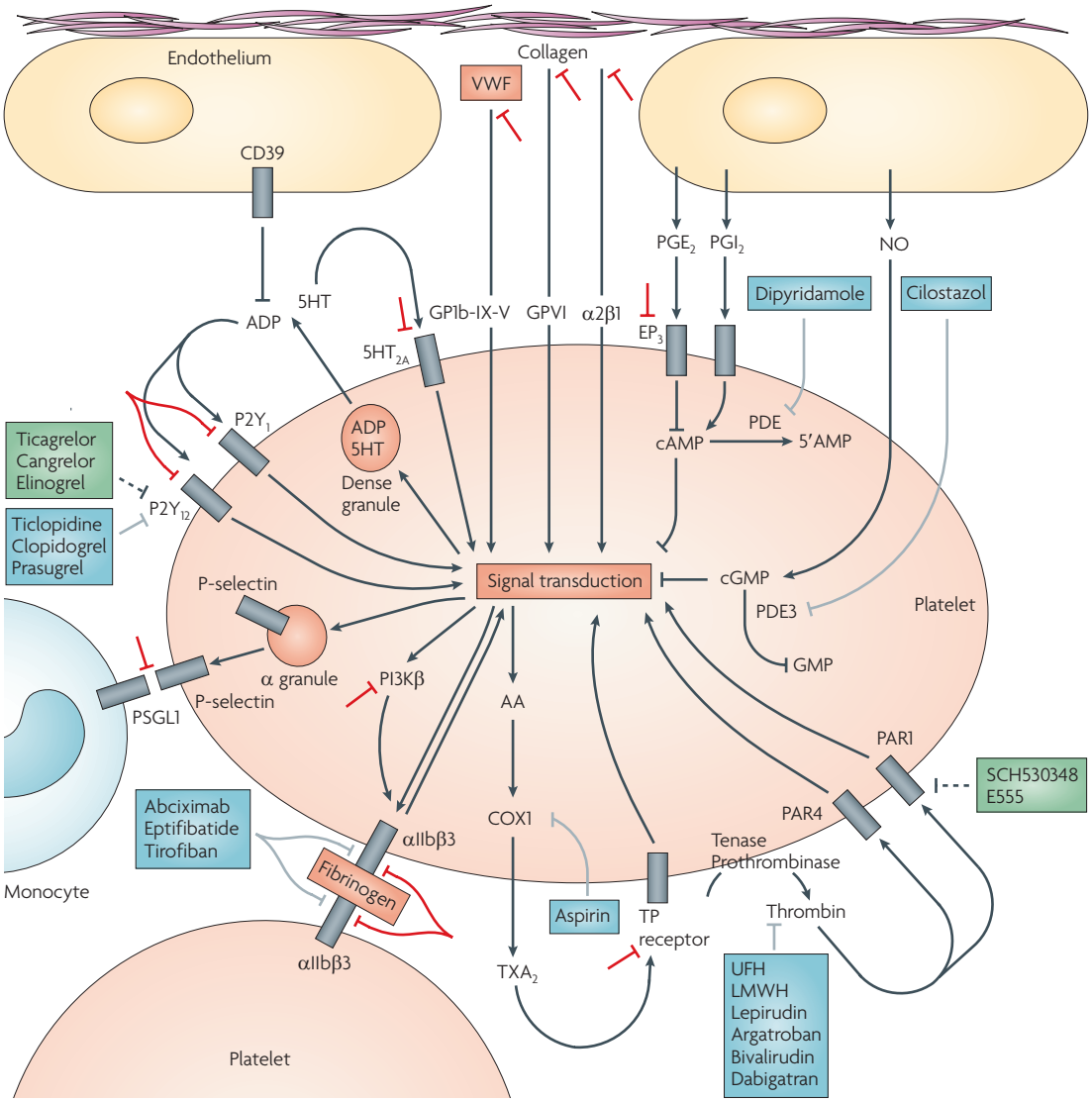


Figure 1 | **Platelet function and molecular targets of antiplatelet agents.** Initial platelet adhesion to damaged vessel walls is mediated by the binding of exposed collagen to platelet surface glycoprotein VI (GPVI) and integrin α2β1 and by the binding of von Willebrand factor (VWF) to the platelet surface glycoprotein 1b (GP1b)-IX-V complex. This complex is also a receptor for other platelet ligands (thrombospondin, collagen and P-selectin), leukocyte integrin αMβ2, and procoagulant factors (thrombin, kininogen, factor XI and factor XII)[147]. Thrombin, generated by the coagulation cascade, is a potent activator of human platelets through two platelet surface receptors: proteinase-activated receptor 1 (PAR1) and PAR4 (REF. 105). Three groups of platelet surface receptors provide important positive feedback loops for platelet activation: P2Y purinoceptor 1 ($P2Y_1$) and $P2Y_{12}$ are stimulated by ADP released from platelet dense granules; 5-hydroxytryptamine 2A receptors ($5HT_{2A}$) are stimulated by 5- hydroxytryptamine (5-HT; also known as serotonin) released from platelet dense granules; and the thromboxane prostanoid (TP) receptor is stimulated by thromboxane A2 (TXA_2) generated by the platelet cyclooxygenase 1 (COX1)-dependent signalling pathway. Platelet-to-platelet aggregation is mediated by fibrinogen and, at high shear flow, by VWF binding to the activated molecular conformation of integrin αIIbβ3, which is the receptor with the highest copy number (~80,000) per platelet[148]. Perpetuation of platelet-to-platelet aggregation is augmented by other receptors, including junctional adhesion molecule A (JAMA) and JAMC, growth-arrest specific gene 6 receptor and ephrin[14]. Platelet–monocyte adhesion is initially mediated by the binding of platelet surface P-selectin (which is only expressed on the platelet surface after platelet degranulation) to its constitutively expressed cognate receptor, P-selectin glycoprotein ligand 1 (PSGL1), on the monocyte surface[149]. Activated platelets, monocytes and microparticles bind coagulation factors and provide a surface for the generation of a fibrin clot[150,151]. Approved antiplatelet agents and their molecular targets are shown in blue. Indirect inhibitors (unfractionated heparin (UFH), low-molecular-weight heparin (LMWH)) and direct inhibitors (lepirudin, argatroban, bivalirudin and dabigatran) of thrombin are not discussed in the main text because, unlike PAR1 antagonists, they are anticoagulants rather than specific antiplatelet drugs. However, their inhibition of thrombin results in reduced platelet activation. Antiplatelet agents in clinical development and their molecular targets are shown in green and investigational strategies for novel antiplatelet agents are shown in red. AA, arachidonic acid; EP_3, prostaglandin E2 receptor EP_3 subtype; NO, nitric oxide; PDE, phosphodiesterase; PG, prostaglandin; PI3Kβ, phosphoinositide 3-kinase β-isoform.

studies provided evidence for an association of laboratory-defined aspirin resistance with a higher risk of recurrent cardiovascular events[20,21]. However, these meta-analyses have important limitations, including: an incidence of aspirin resistance between 5–65% depending on the assay and other variables; the small sample size (n = 14–448); the low number of adverse clinical events, which limited the statistical power of the studies; the occurrence of non-compliance as a confounding variable and the possibility of reporting bias for studies with positive outcomes. Finally, aspirin resistance is in part unrelated to aspirin but is rather the result of underlying platelet hyper-reactivity before the initiation of aspirin therapy[22].

ADP receptor antagonists

ADP, an important platelet agonist *in vivo*, has two types of receptors in the platelet plasma membrane: $P2Y_1$ and $P2Y_{12}$ (REF. 23) (FIG. 1). $P2Y_1$ is a seven-transmembrane domain G protein-coupled receptor, linked to G_q. The result of ADP signalling through the $P2Y_1$ receptor is Ca^{2+} mobilization, a change in platelet shape and rapidly reversible platelet aggregation. $P2Y_{12}$ is also a seven-transmembrane domain receptor, but it is linked to a G_i protein and lowering of cyclic AMP levels. The result of ADP signalling through the $P2Y_{12}$ receptor is the amplification of stable platelet aggregation and secretion[23].

Ticlopidine. Ticlopidine (Ticlid; Roche) is a thienopyridine (TABLE 1) that is metabolized by cytochrome P450 in the liver[24,25]. An active metabolite rather than the parent molecule irreversibly antagonizes the $P2Y_{12}$ receptor (FIG. 1). Ticlopidine is given orally twice a day and was the first $P2Y_{12}$ antagonist to be approved by the US Food and Drug Administration (FDA). However, ticlopidine has been largely replaced in clinical practice by clopidogrel (Plavix; Bristol–Myers Squibb/Sanofi–Aventis), owing to its improved side-effect profile — for example, less neutropaenia and a lower incidence of the rare but dangerous thrombotic thrombocytopaenic purpura[25,26].

Clopidogrel. Clopidogrel is also a thienopyridine (TABLE 1) that is metabolized by cytochrome P450 in the liver and the active metabolite of which irreversibly antagonizes the $P2Y_{12}$ receptor[24,25] (FIG. 1). It is given orally once daily. The clinical benefit of adding clopidogrel to aspirin therapy has been demonstrated by large, multicenter, randomized, controlled trials: CURE (Clopidogrel in Unstable angina to prevent Recurrent Events)[27] in patients with acute coronary syndrome (ACS), unstable angina, or non-ST elevation myocardial infarction; PCI-CURE (Percutaneous Coronary Intervention CURE)[28] and CREDO (Clopidogrel for the Reduction of Events During Observation)[29] in patients undergoing PCI; COMMIT (Clopidogrel and Metoprolol in Myocardial Infarction Trial)[30] and CLARITY-TIMI 28 (Clopidogrel as Adjunctive Reperfusion Therapy–Thrombolysis In Myocardial Infarction 28)[31] in patients with ST-elevation myocardial infarction. However, in patients with stable cardiovascular disease or asymptomatic patients with multiple cardiovascular risk factors, the CHARISMA (Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance) trial[32] demonstrated that the combination of clopidogrel plus aspirin was not significantly more effective than aspirin alone in reducing the rate of myocardial infarction, stroke or death from cardiovascular causes. Furthermore, the risk of moderate-to-severe bleeding was increased[32]. In a retrospective analysis of the CHARISMA trial, dual antiplatelet therapy with clopidogrel and aspirin in the primary prevention subgroup of patients was associated with an increase in cardiovascular death[33]. The cause of this apparent harm has not been elucidated.

Monitoring of clopidogrel by platelet function assays (BOX 1) reveals interpatient response variability[19]. Furthermore, there is evidence that a poor response in such *in vitro* assays (that is, clopidogrel resistance or hyporesponsiveness) is associated with a poor clinical response to clopidogrel, as evidenced by major adverse clinical events[34]. Thus, clopidogrel hyporesponsiveness — as defined by ADP-induced turbidometric platelet aggregation[35–40], ADP-induced platelet aggregation measured by impedance[41], VerifyNow P2Y12 Assay (a point-of-care device that measures ADP-induced platelet aggregation)[42], phosphorylation of vasodilator-stimulated phosphoprotein (VASP)[36,43,44] and the thromboelastogram PlateletMapping System[39] — has been reported to be associated with major adverse clinical events post-PCI. However, the number of adverse clinical events was low in these studies.

Mechanisms of clopidogrel response variability can be classified into broad categories of clinical, genetic and cellular factors[45], of which five are of particular interest. First, patient non-compliance[46,47] may incorrectly suggest a patient is resistant to clopidogrel. Second, drugs that are, like clopidogrel, metabolized by cytochrome P450 in the liver may reduce the effectiveness of clopidogrel. One example of this phenomenon is proton pump inhibitors, although this may be specific to omeprazole[48–51]. However, more recent data suggest that this effect is not clinically significant for any proton pump inhibitor[52]. Third, cigarette smoking increases clopidogrel's effectiveness, presumably by induction of cytochrome P450 1A2 (CYP1A2), a metabolic activator of clopidogrel[53,54]. Fourth, a common reduced-function isoform of CYP2C19 (which occurs in ~30% of individuals of European ancestry, 40% of individuals of African ancestry and >50% of individuals of Asian ancestry) results in significantly lower levels of the active metabolite of clopidogrel, with resultant diminished platelet inhibition and a higher rate of major adverse cardiovascular events[55–57]. Fifth, there is evidence that the pre-clopidogrel response to ADP (the agonist effect that is blocked by clopidogrel) predicts the post-clopidogrel response to ADP, as determined by numerous platelet function assays[58–63]. These data show that the variability in response is caused in part by the platelet response to ADP rather than solely by the platelet response to clopidogrel.

Platelet aggregation
The process by which platelets adhere to one another, mediated by integrin αIIbβ3.

Table 1 | **FDA-approved antiplatelet agents**

Drug*	Mechanism of action	Route of administration	Frequency	Side effects	Limitations	Refs
Aspirin	Irreversible acetylation of Ser529 of cyclooxygenase 1	Oral	Daily	• Bleeding • Gastrointestinal toxicity: heartburn, indigestion, nausea, vomiting and gastric ulceration	• Weak antiplatelet agent	1,15–22, 152
Ticlopidine (Ticlid; Roche)	The active metabolite irreversibly inhibits $P2Y_{12}$ receptors	Oral	Twice daily	• Bleeding • Gastrointestinal toxicity: heartburn, indigestion, nausea and vomiting • Rash • Neutropaenia • Thrombotic thrombocytopaenic purpura (rare)	• More side effects than clopidogrel	24,25
Clopidogrel (Plavix; Bristol–Myers Squibb/ Sanofi–Aventis)	The active metabolite irreversibly inhibits $P2Y_{12}$ receptors	Oral	Daily	• Bleeding • Rash • Neutropaenia • Thrombotic thrombocytopaenic purpura (rare)	• Patient-to-patient variability in response	24,25, 27–44, 48–51, 53–62,64
Prasugrel (Effient; Lilly/ Daiichi Sankyo)	The active metabolite irreversibly inhibits $P2Y_{12}$ receptors	Oral	Daily	• Bleeding	• More haemorrhagic side effects and greater cost than clopidogrel • Contraindicated in patients with a history of stroke or transient ischaemic attacks • Not recommended in patients >75 years old unless they are at high risk of cardiovascular events	65–73
Abciximab (ReoPro; Lilly)	Integrin αIIbβ3 antagonist	Intravenous	Once	• Bleeding • Thrombocytopaenia EDTA-induced pseudo-thrombocytopaenia	• Requires intravenous administration	84,86–91
Eptifibatide (Integrilin; Millennium Pharmaceuticals/ Shering–Plough)	Integrin αIIbβ3 antagonist	Intravenous	Once	• Bleeding • Thrombocytopaenia EDTA-induced pseudothrombocyto-paenia	• Requires intravenous administration	84,86, 89–91
Tirofiban (Aggrastat; Merck)	Integrin αIIbβ3 antagonist	Intravenous	Once	• Bleeding • Thrombocytopaenia EDTA-induced pseudo-thrombocytopaenia	• Requires intravenous administration	84,86, 89–91
Dipyridamole (Boehringer Ingelheim)	Antiplatelet and vasodilatory effects through inhibition of cyclic nucleotide phosphodiesterase- and of adenosine uptake	Oral	Two or three times daily	• Headache • Dizziness • Hypotension and blood pressure lability • Flushing • Gastrointestinal toxicity: nausea, vomiting, diarrhoea and abdominal pain • Rash	• Benefit is most evident in combination with low-dose aspirin	17,97, 99,100
Cilostazol (Pletal; Otsuka)	Antiplatelet and vasodilatory effects through inhibition of cyclic nucleotide phosphodiesterase 3	Oral	Twice daily	• Bleeding • Headache • Diarrhoea • Palpitations • Dizziness • Rash • Pancytopaenia	• Side effects lead to discontinuation of the drug in ~15% of patients	101–103

EDTA, ethylenediaminetetra-acetic acid; FDA, US Food and Drug Administration; ND, not determined; $P2Y_{12}$, P2Y purinoceptor 12; *For chemical structures, see Supplementary information S1 (figure).

Continuing interrelated challenges of clopidogrel treatment include the phenomenon of clopidogrel hyporesponsiveness discussed above, the relatively slow onset of action of clopidogrel compared with aspirin and novel $P2Y_{12}$ antagonists[25] and the ongoing incidence of ischaemic events, including stent thrombosis, in patients treated with clopidogrel and aspirin[64]. As discussed below, this has led to the development of novel $P2Y_{12}$ antagonists and other antiplatelet agents, which are in various phases of investigation (TABLE 2).

Prasugrel. Prasugrel (Effient; Lilly/Daiichi Sankyo) is an orally administered thienopyridine prodrug that, like clopidogrel, is metabolized by liver cytochrome P450 (REF. 65) (TABLE 1) to the active metabolite, which irreversibly inhibits the platelet $P2Y_{12}$ receptor (FIG. 1). However, metabolism of prasugrel to its active metabolite is much more efficient than that of clopidogrel, because it is metabolized by esterases and is less dependent on cytochrome P450 enzymes[66]. As a result, a loading dose of 60 mg prasugrel results in a more rapid, potent and consistent inhibition of platelet function than the standard clopidogrel loading dose of 300 mg[67,68] and the increasingly used clopidogrel loading dose of 600 mg[69,70]. Furthermore, a maintenance dose of prasugrel 10 mg daily results in a more potent and consistent inhibition of platelet function than both the standard clopidogrel maintenance dose of 75 mg daily[69,71] and clopidogrel 150 mg daily[70].

TRITON-TIMI 38 (Trial to assess Improvement in Therapeutic Outcomes by optimizing platelet inhibition with prasugrel–Thrombolysis In Myocardial Infarction 38) was a Phase III trial involving 13,608 patients with acute coronary syndromes and scheduled PCI. Results from the trial demonstrated that prasugrel (60 mg loading dose and a 10 mg daily maintenance dose), as compared with approved doses of clopidogrel (300 mg loading dose and a 75 mg daily maintenance dose), significantly reduced rates of ischaemic events, including stent thrombosis, but with an increased risk of major bleeding, including fatal bleeding[72]. The primary efficacy end point occurred in 12.1% of patients receiving clopidogrel and 9.9% of patients receiving prasugrel. In the prasugrel group, there were also significant reductions in the rates of myocardial infarction, urgent need for PCI, and stent thrombosis. Major bleeding was observed in 2.4% of patients receiving prasugrel and in 1.8% of patients receiving clopidogrel. The rate of life-threatening bleeding was also greater in the prasugrel group, including non-fatal bleeding and fatal bleeding.

A post-hoc subgroup exploratory analysis of the data identified three subgroups of interest that experienced less clinical efficacy and had greater absolute levels of bleeding than the overall cohort, resulting in less net clinical benefit (in patients with age >75 years, body weight <60 kg) or in clinical harm (in patients with a history of stroke or transient ischaemic attack)[72]. The TRITON-TIMI 38 platelet substudy showed that prasugrel causes greater inhibition of ADP-mediated platelet function than clopidogrel in patients with ACS, supporting the hypothesis that greater platelet inhibition leads to a lower incidence of ischaemic events and more bleeding both early and late following PCI[71]. In a prespecified TRITON-TIMI 38 study of 3,534 ST elevation myocardial infarction patients undergoing PCI, prasugrel was more effective than clopidogrel for prevention of ischaemic events, without an apparent excess in bleeding[73]. In TRITON-TIMI 38, subjects with diabetes had a greater prasugrel-induced reduction in ischaemic events than non-diabetic subjects without an observed increase in major bleeding[74].

Largely based on the TRITON-TIMI 38 trial, prasugrel is now approved in the United States and Europe for the prevention of atherothrombotic events in patients with ACS undergoing PCI. An additional Phase III clinical trial (TRILOGY ACS; see Further information) of prasugrel is in progress in the setting of medically managed ACS rather than the PCI in ACS setting of TRITON-TIMI 38. In TRILOGY ACS, the prasugrel dose is 5 or 10 mg daily, depending on the age and weight of the patient.

Ticagrelor. Ticagrelor is an investigational $P2Y_{12}$ antagonist (FIG. 1; TABLE 2). In contrast to ticlopidine, clopidogrel and prasugrel, ticagrelor is a cyclo-pentyl-triazolo-pyrimidine and a direct and reversible $P2Y_{12}$ antagonist[24,25,75]. Like prasugrel, ticagrelor acts more rapidly and is a more potent inhibitor of platelets than clopidogrel and did not significantly increase major bleeding compared with clopidogrel in Phase II studies[76–78]. However, the occurrence of dyspnoea and ventricular pauses were greater, in an apparently dose-dependent manner, in patients receiving ticagrelor than in patients receiving clopidogrel in these studies. Ticagrelor is given orally twice a day.

PLATO (Platelet inhibition and patient Outcomes) was a multicenter, double-blind, randomized trial comparing ticagrelor (180 mg loading dose, 90 mg twice daily thereafter) and clopidogrel (300–600 mg loading dose, 75 mg daily thereafter) for the prevention of cardiovascular events in 18,624 patients admitted to hospital with an ACS, with or without ST-segment elevation[79]. At 12 months, the primary end point — a composite of death from vascular causes, myocardial infarction, or stroke — had occurred in 9.8% of patients receiving ticagrelor, compared with 11.7% of those receiving clopidogrel (hazard ratio: 0.84; 95% confidence interval: 0.77–0.92; $P < 0.001$).

Predefined hierarchical testing of secondary end points showed significant differences in the rates of other composite end points, as well as myocardial infarction alone (5.8% in the ticagrelor group versus 6.9% in the clopidogrel group; $P = 0.005$) and death from vascular causes (4.0% versus 5.1%; $P = 0.001$) but not stroke alone (1.5% versus 1.3%; $P = 0.22$). The rate of death from any cause was also reduced with ticagrelor (4.5% versus 5.9% with clopidogrel; $P < 0.001$). No significant difference in the rates of major bleeding was found between the ticagrelor and clopidogrel groups (11.6% and 11.2%, respectively; $P = 0.43$), but ticagrelor was associated with a higher rate of major bleeding not related to coronary artery bypass grafting (4.5% versus 3.8%; $P = 0.03$), including more instances of fatal intracranial bleeding and fewer of fatal bleeding of other types[79].

Box 1 | **Monitoring of antiplatelet drugs**

There is a delicate balance between thrombosis and haemorrhage. Antiplatelet therapy decreases the risk of thrombosis but, unsurprisingly, increases the risk of bleeding. An important question is whether or not monitoring antiplatelet drugs in patients, with resultant treatment modifications, would be clinically useful in the reduction of thrombotic and/or haemorrhagic risk in individual patients.

Monitoring of aspirin therapy can be accomplished by: assays that use thromboxane as the end point (serum thromboxane B_2 and urinary 11-dehydro thromboxane B_2); assays that use arachidonic acid as the stimulus (turbidometric platelet aggregometry, whole-blood impedance platelet aggregometry (for example, Multiplate and Dynabyte), VerifyNow Aspirin Assay, measurement of platelet surface P-selectin, platelet surface activated integrin αIIbβ3 (also known as GPIIb–IIIa) and leukocyte–platelet aggregates, TEG PlateletMapping system (Haemoscope, Impact cone and plate(let) analyser)); or other assays (PFA-100)[139].

Monitoring of therapy with clopidogrel (or other P2Y purinoceptor 12 ($P2Y_{12}$) antagonists) can be accomplished by assays that use ADP as the stimulus: vasodilator-stimulated phosphoprotein (VASP) phosphorylation, turbidometric platelet aggregometry, whole-blood impedance platelet aggregometry (for example, Multiplate), the VerifyNow P2Y12 Assay, measurement of platelet surface P-selectin, platelet surface activated integrin αIIbβ3 and leukocyte–platelet aggregates, the TEG PlateletMapping system, or the Impact cone and plate(let) analyser[139].

Point-of-care assays (also referred to as point-of-service assays) have potentially great advantages — for example, to help immediate decision-making about the type and dose of antiplatelet therapy in the interventional cardiology suite. A rigorous definition of a point-of-care assay is one that meets all of the following criteria: use at or near the patient bedside; easy to use without special skills; no sample processing; no pipetting; and a rapid readout. The only currently available device that meets these criteria is VerifyNow[42,140], but other devices are in development.

Single nucleotide polymorphisms (SNPs) are an important variable in the response of platelets to antiplatelet therapy[141] — as has been most clearly shown for $P2Y_{12}$ antagonists. Carriers of the common gene variant encoding a reduced-function cytochrome P450 2C19 (CYP2C19) have lower levels of the active metabolite of clopidogrel, diminished platelet inhibition and a higher rate of major adverse clinical events than non-carriers[55–57]. By contrast, prasugrel, because of its different metabolism (by esterases and with less dependence upon CYP enzymes), is unaffected by the reduced-function *CYP2C19* allele[142,143]. Ticagrelor and cangrelor are unaffected because they do not require metabolism.

Meta-analyses suggest that assays of the effects of aspirin and clopidogrel on platelet function can be used to predict clinical outcomes[20,21,34]. However, the benefit of changing treatment based on platelet function tests and/or SNPs remains unproven. Is aspirin and/or clopidogrel hyporesponsiveness an inherent, non-modifiable risk marker or a modifiable risk factor? Will antiplatelet agents under development (for example, prasugrel, ticagrelor and cangrelor) obviate the need for platelet function testing and/or SNP profiling because of their greater and more consistent inhibition of platelet function, or will they result in a greater need for platelet function testing because of their increased risk for haemorrhage? Small published studies suggest that modifying clopidogrel dose based on the VASP assay[44,144], or adding an integrin αIIbβ3 antagonist based on light transmission aggregometry[145] or the VerifyNow assay[146] may be of clinical benefit. However, large randomized studies of guided therapy are needed to resolve these questions, and several are in progress: GRAVITAS, 3T/2R, the DANTE trial, TRIGGER-PCI and the ARTIC study (see Further information).

Cangrelor. Cangrelor is an investigational, direct-acting, reversible $P2Y_{12}$ antagonist[24,25] (FIG. 1; TABLE 2). Unlike the $P2Y_{12}$ antagonists described above, cangrelor is administered intravenously, and its effects are rapidly reversed after the end of the infusion. Like prasugrel and ticagrelor, cangrelor results in a more rapid onset of action and greater degree of platelet inhibition than clopidogrel, and showed no significant increase in major bleeding compared with clopidogrel in Phase II studies[80,81]. Cangrelor underwent two Phase III trials (CHAMPION-PCI and CHAMPION-PLATFORM; see Further information), which were stopped early owing to lack of efficacy (see the Medicines Company news release). Cangrelor is still being studied as a 'bridge' for patients on clopidogrel who need to terminate treatment before surgery (the BRIDGE study; see Further information).

Elinogrel. Elinogrel is an investigational, direct-acting, reversible $P2Y_{12}$ antagonist with a novel structure[82] (FIG. 1; TABLE 2). It can be administered orally or intravenously and is currently undergoing Phase II trials (A study to determine the pharmacokinetics of elinogrel in healthy volunteers and patients with mild, moderate and severe renal impairment; see Further information).

Combined $P2Y_1$ and $P2Y_{12}$ antagonists. Preliminary *in vitro* evidence has indicated that the modified diadenosine tetraphosphonate derivatives diadenosine P1,P4-tetraphosphate (Ap_4A), P1,P4-dithio ($ApSp_2pSA$), chloromethyl (Ap_2CHClp_2A) and dithio-chloromethyl (ApSpCHClppSA) synergistically inhibit platelet activation through both $P2Y_1$ and $P2Y_{12}$ (REF. 83) (BOX 2; FIG. 1). The ADP-stimulated $P2Y_1$-mediated increase in platelet cytosolic Ca^{2+} and $P2Y_{12}$-mediated decrease in phosphorylated VASP were measured by whole-blood flow cytometry. ADP-stimulated platelet aggregation, mediated by $P2Y_1$ and $P2Y_{12}$, was measured optically. Ap_4A inhibited $P2Y_1$ function, and this was enhanced with $ApSp_2pSA$ and reduced with Ap_2CHClp_2A. Ap_4A and $ApSp_2pSA$ weakly inhibited $P2Y_{12}$ whereas Ap_2CHClp_2A and ApSpCHClppSA were potent inhibitors. The half-maximal inhibitory concentration (IC_{50}) values for inhibition of platelet aggregation by Ap_4A and its derivatives were much lower than the IC_{50} values for inhibition of the ADP-induced increase in cytosolic Ca^{2+} or decrease in VASP phosphorylation. Inhibition of platelet aggregation was greatest with ApSpCHClppSA. Thus, Ap_4A and its derivatives inhibit ADP-induced platelet activation through both $P2Y_1$ and $P2Y_{12}$, and this dual inhibition may be synergistic. These compounds, especially ApSpCHClppSA, may therefore have utility as antithrombotic agents.

Integrin αIIbβ3 antagonists

The three FDA-approved integrin αIIbβ3 antagonists are abciximab (ReoPro; Lilly), eptifibatide (Integrilin; Millennium Pharmaceuticals/Schering–Plough), and tirofiban (Aggrastat; Merck)[84] (FIG. 1; TABLE 1). Evolving out of the discovery that integrin αIIbβ3 was deficient in patients with an inherited platelet aggregation defect[85], integrin αIIbβ3 antagonists were the first rationally designed antiplatelet therapy[86]. Abciximab is a murine human chimeric F_{ab} fragment that was derived from the murine monoclonal antibody 7E3, eptifibatide is a KGD-containing cyclic heptapeptide and tirofiban is a non-peptide derivative based on the RGD sequence[84] (TABLE 1). All three drugs are administered intravenously.

The primary mechanism of action of the integrin αIIbβ3 antagonists is not inhibition of platelet activation but of the final common pathway of platelet-to-platelet aggregation: fibrinogen, or under high shear stress, VWF binding to integrin αIIbβ3 (FIG. 1). In addition to inhibiting platelet aggregation, integrin αIIbβ3 antagonists have a specific anticoagulant action, as evidenced by prolongation of the activated clotting time[87], inhibition of thrombin generation[88] and inhibition of platelet procoagulant activity[89]. The mechanisms of this anticoagulant effect of integrin αIIbβ3 antagonists include inhibition of prothrombin binding to αIIbβ3 (REF. 90), with less resultant thrombin generation and inhibition of procoagulant platelet-derived microparticle formation[88]. Abciximab also binds to integrins αvβ3 (the vitronectin receptor) and αMβ2 (also known as MAC1 and CD11b–CD18)[86], but the clinical significance of this binding remains unclear.

Based on numerous large clinical trials reviewed elsewhere[84], the FDA approved the use of integrin αIIbβ3 antagonists for PCI (abciximab and eptifibatide) and ACS (eptifibatide and tirofiban). However, except in high-risk patients, the clinical use of integrin αIIbβ3 antagonists has recently decreased in inverse proportion to the increased use of ADP receptor antagonists, owing to the demonstrated benefit of ADP receptor antagonists[91].

Attempts to develop oral integrin αIIbβ3 antagonist therapy have been unsuccessful[92]. One possible explanation for this failure is that integrin αIIbβ3 may remain in its high-affinity conformation after dissociation of the antagonist[93], thereby facilitating the binding of fibrinogen and/or VWF with a resultant paradoxical predisposition to thrombosis. Thus, because integrin αIIbβ3 antagonists do not inhibit platelet activation, they may result in more activated platelets in the circulation, which may be procoagulant as a result of, for example, phosphatidylserine expression following platelet activation and more platelet–leukocyte interactions. Preliminary studies have been performed to identify an integrin αIIbβ3 antagonist with a diminished capacity to induce these possibly disadvantageous conformational changes in integrin αIIbβ3 (REFS 94,95) (BOX 2; FIG. 1).

For example, 33,264 small molecules were tested for their ability to inhibit the adhesion of washed platelets to immobilized fibrinogen[94], of which 102 compounds exhibited 50% or more inhibition. One of these (RUC-1 at 265 g per mol) inhibited ADP-induced platelet aggregation (IC_{50}: 13 ± 5 μM), the binding of soluble fibrinogen to platelets induced by monoclonal antibody AP5, and the binding of soluble fibrinogen and a cyclic RGD peptide to purified integrin αIIbβ3. RUC-1 did not affect the function of GP1b, α2β1 or the other β3 family receptor, αVβ3. Molecular docking simulations suggested that RUC-1 interacts with αIIb but not β3. RUC-1 induced partial exposure of an αIIb ligand-induced binding site (LIBS), but did not induce exposure of two β3 LIBS. Transient exposure of purified αIIbβ3 to eptifibatide, but not RUC-1, enhanced fibrinogen binding ('priming'). Thus, RUC-1 provides a prototype for small-molecule selective inhibition of αIIbβ3, without receptor priming, through targeting of αIIb.

A single-chain antibody specific for a LIBS ($scFv_{anti-LIBS}$) was cloned and genetically fused with a potent, direct factor Xa inhibitor, tick anticoagulant peptide (TAP)[96]. Specific antibody binding of the fusion molecule $scFv_{anti-LIBS}$–TAP was shown by flow cytometry, and inhibition of factor Xa was demonstrated in chromogenic assays. *In vivo* anticoagulant efficiency was determined by Doppler flow in a ferric chloride-induced carotid artery thrombosis model in mice. The prolongation of occlusion by $ScFv_{anti-LIBS}$–TAP was comparable to that caused by enoxaparin, recombinant TAP and non-targeted mutant scFv–TAP. $ScFv_{anti-LIBS}$–TAP caused antithrombotic effects at low doses, at which the non-targeted mutant scFv-TAP did not have an effect. In contrast to the other anticoagulants tested, bleeding times were not prolonged by $scFv_{anti-LIBS}$–TAP. Thus, this novel, clot-targeting approach of anticoagulants through a single-chain antibody directed against a LIBS epitope on integrin αIIbβ3 may result in effective anticoagulation with reduced bleeding risk.

Phosphodiesterase inhibitors

Dipyridamole is a pyrimidopyrimidine derivative with antiplatelet and vasodilator properties[97] (TABLE 1). The antiplatelet effects of dipyridamole have been reported to be due to several mechanisms of action, including inhibition of cyclic nucleotide phosphodiesterase and blockade of adenosine uptake — both of which result in increased intraplatelet cAMP levels, which inhibits signal transduction[97] (FIG. 1). A modified-release formulation of 200 mg dipyridamole with improved bioavailability has been developed in association with low-dose (25 mg) aspirin (Aggrenox; Boehringer Ingelheim)[17,97]. Aggrenox is FDA-approved for stroke prevention based on the results of the European Stroke Prevention Study 2 (ESPS-2)[98] and the European Stroke Prevention Reversible Ischemia Trial (ESPRIT)[99]. However, Aggrenox was not superior to clopidogrel in the treatment of recurrent stroke in the recently completed Prevention Regimen for Effectively Avoiding Second Strokes (PROFESS) trial[100].

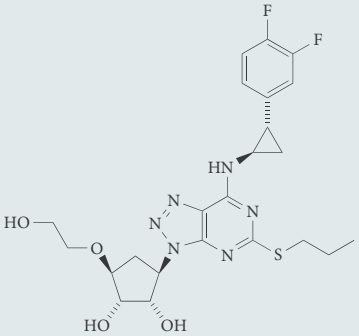
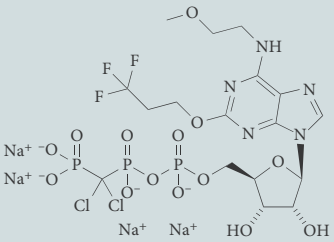
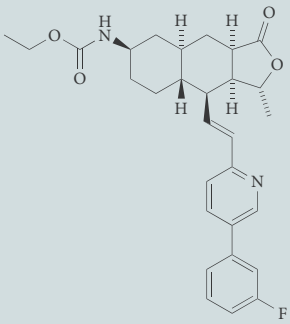
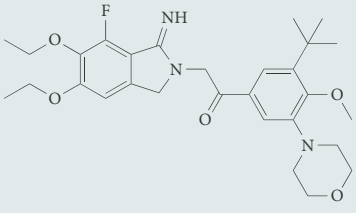
Cilostazol (Pletal; Otsuka) is an oral selective cyclic nucleotide phosphodiesterase 3 (PDE3) inhibitor with antiplatelet, vasodilatory and antimitogenic effects[101] (FIG. 1; TABLE 1). It is FDA-approved for the treatment of intermittent claudication, and has been investigated for use in PCI and stroke. Cilostazol, in addition to aspirin and clopidogrel, seems to be effective and safe in reducing the risk of restenosis and repeat revascularization after PCI, but available evidence is limited by the small size of the observed effects[102]. The side effects of cilostazol (including headaches, gastrointestinal symptoms and skin rash) cause ~15% of patients to discontinue the drug[103,104].

PAR1 antagonists

Thrombin, generated by the coagulation cascade, is a potent activator of human platelets through actions on two platelet surface G protein-coupled receptors: PAR1 and PAR4 (REF. 105) (FIG. 1). PAR1 antagonists are therefore inhibitors of thrombin-induced platelet activation

but not thrombin-induced cleavage of fibrinogen — the final step in coagulation. SCH 530348, a synthetic tricyclic 3-phenylpyridine analogue of himbacine, is an orally administered, rapidly absorbed, high-affinity reversible PAR1 antagonist (FIG. 1; TABLE 2). A Phase II trial demonstrated that SCH 530348 inhibited PAR1 thrombin receptor activating peptide (TRAP)-induced platelet aggregation in a dose-dependent manner, was generally well tolerated and did not cause an increase in major bleeding, even when administered with aspirin and clopidogrel[106]. Two Phase III trials of SCH 530348 are currently in progress: TRA-CER and TRA 2P-TIMI

Table 2 | **Antiplatelet agents in clinical development**

Drug	Structure	Mechanism of action	Route of administration	Frequency	Trial Phase	Refs
Ticagrelor	 Cyclo-pentyl-triazolo-pyrimidine	Reversible inhibition of $P2Y_{12}$ receptors	Oral	Twice daily	III	24,25, 75–78
Cangrelor	 ATP analogue	Reversible inhibition of $P2Y_{12}$ receptors	Intravenous	ND	III	24,25, 80,81
Elinogrel	Structure not published	Reversible inhibition of $P2Y_{12}$ receptors	Oral or intravenous	ND	II	82
SCH 530348	 Tricyclic 3-phenylpyridine analogue of himbacine	Reversible inhibition of PAR1	Oral	Daily	III	106
E5555	 2-Iminopyrrolidine antagonist	Reversible inhibition of PAR1	Oral	Daily	II	107

ND, not determined; PAR1, proteinase-activated receptor 1; $P2Y_{12}$, P2Y purinoceptor 12.

50 (see Further information). E5555 is another PAR1 antagonist[107] (FIG. 1; TABLE 2) that is currently undergoing Phase II trials (A double-blind study of E5555 in Japanese patients with acute coronary syndrome; Safety and tolerability of E5555 and its effects on markers of intravascular inflammation in subjects with acute coronary syndrome; Safety and tolerability of E5555 and its effects on markers of intravascular inflammation in subjects with coronary artery disease; see Further information).

Other investigational approaches

GPVI antagonists. Platelet surface GPVI, which is complexed with the Fc receptor γ-chain, is an important receptor for platelet adhesion to exposed collagen[11] (FIG. 1). GPVI antagonists (BOX 2; FIG. 1) that are under investigation as antiplatelet agents include a snake venom metalloproteinase (kistomin)[108] and a human GPVI-specific monoclonal antibody that results in cAMP-dependent endocytosis of the GPVI–Fc receptor γ-chain complex[109]. This approach may have advantages by blocking the collagen GPVI-dependent initiation of thrombosis under conditions of high shear stress such as arterial stenosis.

The GP1b-cleaving snake venom metalloproteinase kistomin has been reported to inhibit collagen-induced platelet aggregation[108]. Moreover, kistomin inhibited platelet aggregation induced by convulxin (a GPVI agonist) and a GPVI-specific antibody in a concentration- and time-dependent manner. Kistomin treatment decreased the levels of platelet GPVI but not integrin α2β1 and αIIbβ3, accompanied by the formation of GPVI cleavage fragments. In addition, intact platelet GPVI and recombinant GPVI were digested by kistomin to release 25- and 35-kDa fragments, suggesting that kistomin cleaved GPVI near the mucin-like region. Four synthetic peptides ranging from Leu180 to Asn249 were designed as substrates for kistomin, and kistomin cleaved these synthetic peptides at FSE205–A206TA and NKV218–F219TT. In addition, GPVI-specific antibody-induced tyrosine kinase phosphorylation in platelets was reduced after kistomin pretreatment, and platelet adhesion to collagen but not to fibrinogen was attenuated by kistomin. These studies provided the first evidence that a snake venom metalloproteinase, kistomin, inhibits the interaction between collagen and platelet GPVI through its proteolytic activity on GPVI, and thus suggest an alternative strategy for developing new antithrombotic agents.

GPVI-specific autoantibodies from the first reported patient with ongoing platelet GPVI deficiency caused by these antibodies have been characterized[109]. To obtain experimentally useful human GPVI-specific monoclonal antibodies with characteristics similar to the GPVI-specific autoantibodies, human GPVI-specific mouse monoclonal antibodies were generated and two representative antibodies, mF1201 and mF1232, were selected. The binding of these selected antibodies to GPVI was inhibited by the autoantibodies. *In vitro*, mF1201 but not mF1232 induced human platelet activation and GPVI shedding, and mF1232 inhibited collagen-induced human platelet aggregation. Administration of mF1201 to monkeys caused immunodepletion of GPVI with significant thrombocytopaenia and GPVI shedding, but this was not the case with mF1232.

When a human–mouse chimeric form of mF1232 (cF1232) was labelled with a fluorescent endocytosis probe and administered to monkeys, fluorescence increased in circulating platelets and surface GPVI was lost[109]. Loss of platelet surface GPVI mediated by cF1232 was successfully reproduced *in vitro* in the presence of a cAMP-elevating agent. Thus, these studies characterize a cAMP-dependent endocytosis of GPVI mediated by a human GPVI-specific monoclonal antibody, which may be the basis for a novel antiplatelet therapy. In the potential clinical application of cF1232, its administration by a subcutaneous injection route would be more practical and useful than an intravenous route, to maintain a longer effect of GPVI immunodepletion. A single subcutaneous injection of cF1232 into monkeys led to sustained low titre plasma concentrations and long-term antiplatelet effects due to immunodepletion of GPVI without significant thrombocytopaenia[109].

Integrin α2β1 antagonists. Integrin α2β1, a collagen receptor (FIG. 1) is a good candidate for antithrombotic therapy as its overexpression is associated with stroke and myocardial infarction and its underexpression results in a mildly prolonged bleeding time without a profound bleeding disorder[110]. Structure–activity studies have identified potent and selective small-molecule inhibitors (BOX 2; FIG. 1), one of which has shown *in vivo* efficacy for inhibition of this platelet receptor in an animal model of arterial thrombosis. These results suggest that targeting integrin α2β1 could potentially be a safe, effective approach to long-term therapy for cardiovascular disease.

Serotonin receptor antagonists. Release of serotonin from platelet dense granules with subsequent activation of $5HT_{2A}$ receptors on the platelet surface is a potent augmentative stimulus for platelet aggregation (FIG. 1). However, serotonin receptor antagonists (FIG. 1) have not been successfully exploited as antiplatelet agents, possibly owing to their lack of specificity for the $5HT_{2A}$ receptor subtype. Recently, APD791 has been developed as a potent and highly selective inverse agonist of the $5HT_{2A}$ receptor that displays no functional activity at the $5HT_{2B}$ or $5HT_{2C}$ subtypes[111]. The IC_{50} for inhibition of serotonin-stimulated platelet aggregation is 25 nM for APD791, which is several orders of magnitude lower than the IC_{50} for previously-reported serotonin receptor antagonists such as sarpogrelate (IC_{50} 4.6 μM). In a well-established preclinical canine model of recurrent thrombosis mimicking unstable angina, APD791 selectively inhibited serotonin-mediated platelet activation and attenuated recurrent thrombosis, irrespective of whether it was administered before or after the induced coronary artery injury[111].

NO-releasing variant of aspirin. The addition of a nitric oxide moiety to aspirin, producing a compound termed NCX-4016 (NicOx SA) (BOX 2), has been shown to inhibit thrombosis in an *in vivo* rat model[112]. This effect

Box 2 | **Investigational strategies for novel antiplatelet agents**

- Antagonism of integrin αIIbβ3 (also known as GPIIb–IIIa) with a diminished capacity to induce conformational changes in αIIbβ3 (REFS 94,95)
- Targeting of activated platelets — for example, those with a ligand-induced binding site exposed on αIIbβ3 (REF. 96)
- Glycoprotein VI antagonism[108,109]
- α2β1 antagonism[110]
- Glycoprotein 1b antagonism[114]
- Antagonism of P-selectin– P-selectin ligand 1 signalling[116–118]
- Thromboxane receptor antagonism[123]
- Combined thromboxane receptor and thromboxane synthase antagonism[130]
- 5-hydroxytryptamine 2A receptor antagonism[111]
- Antagonism of the platelet prostaglandin E2 receptor EP_3 subtype[134]
- Antagonism of both P2Y purinoceptor 1 ($P2Y_1$) and $P2Y_{12}$ by modified diadenosine tetraphosphonate derivatives[83]
- Antagonism of the β isoform of phosphoinositide 3-kinase[136]
- Nitric oxide-releasing variant of aspirin[113]

is most likely due to nitric oxide release from the compound, but may also be dependent to some extent on suppression of thromboxane synthesis. It is possible that, in certain circumstances, a dual mechanism of action in inhibiting platelet aggregation (together with the ability of NCX-4016 to inhibit neutrophil adherence) may offer advantages over agents that only inhibit platelet aggregation through a single mechanism. The greater gastric tolerability of NCX-4016 compared with aspirin is also an attractive feature of this compound.

Type 2 diabetes mellitus is known to negatively affect biological properties of venous vasculature and, particularly, to reduce endothelium-derived nitric oxide release. The effects of NCX-4016 on vein grafts of patients with diabetes and control patients undergoing elective coronary artery bypass grafting (CABG) was therefore recently assessed[113]. In 40 patients with ischaemic heart disease (20 with diabetes and 20 without diabetes), the effects of nitric oxide-releasing aspirin (NORA) were tested on segments of saphenous vein conduits that were harvested during elective CABG. Functional responses were tested by exposing vein grafts to NORA and to standard vasoactive agents in an organ bath preparation. Significant impairment of acetylcholine-induced, endothelial-dependent vasodilation was documented in vein grafts of subjects with diabetes. NORA induced a significant and comparable vascular relaxation in all venous segments of patients with and without diabetes (56 ± 12% versus 61 ± 11% of maximal relaxation, respectively). This preliminary study suggests that NORA may be a promising therapy for patients with diabetes undergoing CABG.

GP1b antagonists. Another approach to antiplatelet therapy is antagonism of GP1b (BOX 2; FIG. 1). The F_{ab} fragment of 6B4, a murine monoclonal antibody that targets human GP1bα and blocks the binding of VWF, has antithrombotic properties[114]. A fully recombinant and humanized version, h6B4-F_{ab}, maintains its inhibitory capacities *in vitro* and *ex vivo* after injection into non-human primates. The antithrombotic effect of h6B4-F_{ab} on acute platelet-mediated thrombosis was studied in baboons in which thrombus formation was induced at an injured and stenosed site of the femoral artery, allowing for cyclic flow reductions (CFRs) which were measured on an extracorporeal femoral arteriovenous shunt[114]. Injection of 0.5 mg per kg of h6B4-F_{ab} reduced the CFRs by 80%, and two extra injections, resulting in cumulative doses of 1.5 and 2.5 mg per kg, completely inhibited the CFRs. Further development of this approach is awaited.

Antagonists of P-selectin and PSGL1. Several approaches have been used to antagonize the P-selectin–PSGL1 interaction (BOX 2; FIG. 1), and these approaches may also inhibit the recruitment of microparticles expressing tissue factor to the growing thrombus[115]. There have been investigations to determine whether administration of a soluble recombinant PSGL1 chimera (rPSGL–Ig) in conjunction with thrombolytic therapy would enhance thrombolysis by preventing ongoing interactions of leukocytes with platelets and the injured arterial wall[116]. An occlusive thrombus was formed in an internal iliac artery of Yorkshire pigs by placement of a copper coil in the artery under fluoroscopic guidance. Pigs then received heparin and, 15 minutes later, either vehicle or rPSGL–Ig followed by infusion with 25 mg tissue plasminogen activator according to the 90-minute regimen. Blood flow through the artery was monitored by angiography and scored on a scale of 0–3.

Lysis of the thrombus was accelerated by 70% in pigs treated with rPSGL-Ig 250 mg per kg compared with control. The arteries of eight out of nine control pigs re-occluded after the end of tissue plasminogen activator infusion, whereas no re-occlusion was observed in eight out of nine pigs in the rPSGL–Ig group. When the dose of rPSGL–Ig was increased to 500 mg per kg, time to lysis was shortened by 61% from control. Re-occlusion occurred in 6.0 ± 15.2 minutes in control but not in any rPSGL–Ig-treated pig (n = 5 each). In addition, near-normal flow after thrombolysis was achieved 59% and 58% faster in the two rPSGL–Ig groups than in their respective controls. This study suggests that inhibition of leukocyte accumulation at the site of thrombosis with rPSGL–Ig may represent a safe therapeutic intervention that could be important in accelerating thrombolysis, achieving optimal reperfusion, and reducing incidence of acute re-occlusion.

The *in vitro* activity, pharmacokinetic properties and anti-inflammatory and antithrombotic efficacy of an orally active small-molecule antagonist of P-selectin, PSI-697 (2-(4-chlorobenzyl)-3-hydroxy-7,8,9,10-tetrahydrobenzo[*h*] quinoline-4-carboxylic acid) have been characterized[117]. Biacore and cell-based assays were used to demonstrate the ability of PSI-697 to dose-dependently inhibit the binding of human P-selectin to human PSGL1, inhibiting 50% of binding at 50–125 μM. The pharmacokinetics of PSI-697 in rats was characterized by low clearance, short half-life, low volume of distribution and moderate apparent oral bioavailability. A surgical inflammation model, using

Cyclic flow reductions
Changes in blood flow over time that are dependent on the formation and dissolution of platelet aggregates.

exteriorized rat cremaster venules, showed that PSI-697 reduced the number of rolling leukocytes by 39% versus vehicle control. In a rat venous thrombosis model, PSI-697 reduced thrombus weight by 18% relative to vehicle, without prolonging bleeding time. Finally, in a rat carotid injury model, PSI-697 administered 1 hour before arterial injury and once daily thereafter for 13 days resulted in significant dose-dependent decreases in intima:media ratios of 40.2%:25.7% compared with vehicle controls. These data demonstrate the activity of PSI-697 *in vitro* and after oral administration in animal models of both arterial and venous injury and support the clinical evaluation of this novel antagonist of P-selectin in atherothrombotic and venous thrombotic indications.

Another oral small-molecule inhibitor of P-selectin, PSI-421, was evaluated in a baboon model of stasis-induced deep vein thrombosis (DVT)[118]. Primates received a single dose of either 1 mg per kg PSI-421 orally or 0.57 mg per kg enoxaparin sodium subcutaneously, two days prior to and continued 6 days after thrombosis, or no treatment. PSI-421-treated primates had greater percent vein reopening and less vein wall inflammation than the enoxaparin and controls at day 6. Microparticle tissue factor activity (MPTFA) was significantly lower in the animals receiving PSI-421 immediately after thrombosis (T +6 hours on day 0), suggesting lower potential for thrombogenesis in these animals. PSI-421 also reduced soluble P-selectin levels versus controls at T +6 hours on day 0, day 2 and day 6. This study therefore demonstrated a reduction in MPTFA associated with vein reopening and reduced vein inflammation due to oral P-selectin inhibition in a baboon model of DVT.

Thus, antagonism of the P-selectin–PSGL1 interaction has potential benefit in both arterial and venous thrombosis. Although this approach may have advantages, including an anti-inflammatory effect and less bleeding (because it is not directed against a key molecule in haemostasis), further study is needed to determine the possible clinical benefit.

Thromboxane receptor antagonists. Thromboxane receptor antagonists (BOX 2; FIG. 1) may have pharmacological advantages over aspirin: not only do they block the effect of thromboxane A_2 on platelets, but they also inhibit the deleterious effects of thromboxane receptor ligands such as endoperoxides, prostanoids and isoprostanes[119,120]. They antagonize the effects of thromboxane A_2 on the thromboxane receptor that is present on other cells such as monocytes and vascular cells, and preserve the beneficial COX2-dependent endothelial production of prostacyclin, thereby maintaining the inhibition of platelet aggregation and vasodilation.

S18886 is an oral specific thromboxane receptor antagonist. It has been reported to have an antithrombotic effect in animals that is superior to aspirin and equivalent to clopidogrel[121], and to improve endothelial dysfunction in patients with coronary artery disease receiving aspirin[122]. In a multicenter, double-blind, pharmacokinetic–pharmacodynamic study of S18886, 30 patients with peripheral artery disease were randomized to receive five different oral dosages for 12 weeks[123]. This study identified the minimal effective plasma concentration of S18886 required for potent antiplatelet efficacy in patients with stable peripheral arterial disease. Other thromboxane receptor antagonists include Z-335 (which is in Phase II trials in Japan for chronic arterial occlusive disorders), PBT-3, S145Na, S-1452, SQ30741 and BMS-180291 (REFS 124–129). In the Drug evaluation in Atherosclerotic Vascular disease In Diabetics (DAVID) study, picotamide, a combined inhibitor of thromboxane A_2 synthase and receptor, reduced 2-year mortality in patients with diabetes and peripheral arterial disease[130]. Other combined inhibitors of thromboxane A_2 synthase and the thromboxane receptor include UK-141161, UK-147535, BM-531, BM-573 and BM-591 (REFS 131–133).

Antagonism of the platelet EP_3 receptor for PGE_2. Production of prostaglandin E_2 (PGE_2) by an inflamed plaque exacerbates atherothrombosis and may limit the effectiveness of current therapeutics. Platelets express a G protein-coupled receptor, EP_3, for PGE_2 (FIG. 1). Like the $P2Y_{12}$ receptor for ADP, it inhibits cAMP synthesis, causing platelet activation and aggregation. However, unlike ADP, facilitation of platelet aggregation through the PGE_2–EP_3 pathway is dependent on co-agonists that can mobilize Ca^{2+}.

A ligand-based design strategy was used to develop peri-substituted bicylic acylsulphonamides as potent and selective EP_3 antagonists[134]. DG-041, a selective EP_3 antagonist, inhibited PGE_2-dependent platelet aggregation *in vitro* and *ex vivo*. PGE_2 resensitized platelets to agonist even when the $P2Y_{12}$ receptor was blocked by clopidogrel, and this effect could be inhibited by DG-041. Unlike clopidogrel, DG-041 did not affect the bleeding time in rats, nor was the bleeding time further increased when DG-041 was co-administered with clopidogrel. Thus, antagonism of the platelet EP_3 receptor for PGE_2 might have a superior safety profile to $P2Y_{12}$ antagonists and could represent a novel antiplatelet strategy.

Antagonism of the β isoform of PI3K. PI3Kβ plays a crucial part in shear stress-induced arterial thrombosis[135]. The antithrombotic effects of a PI3K inhibitor that is selective for the β isoform, TGX221, were compared with the effects of non-selective PI3K inhibitors (LY294002 and wortmannin) and a PI3K inhibitor that is selective for the δ isoform (IC87114) in the rat[136]. TGX221 (2.5 mg per kg intravenously) abolished CFRs in a Folts-like carotid artery stenosis preparation of thrombosis while not changing bleeding time, heart rate, blood pressure or carotid vascular conductance. By contrast, although the PI3K non-isoform-selective inhibitor wortmannin (5 mg per kg intravenously) was as effective in abolishing CFRs, it caused marked hypotension and carotid vasodilatation. In isolated mesenteric arteries, wortmannin was the most potent relaxant of K^+-precontracted vessels. LY294002 and TGX221 were 40–60 fold less potent and IC87114 was without effect. These findings suggest that the β isoform of PI3K is crucial for the selective development of arterial thrombosis *in vivo*. The multiple actions

of wortmannin are consistent with inhibition of the PI3K C2 domain-containing α-polypeptide (PI3KC2α) and PI3KC2β isoforms and possibly other actions. Thus, a selective inhibitor of the β isoform of PI3K offers advantages as a potential approach to the treatment of thrombosis without unwanted extension of bleeding time or adverse cardiovascular sequelae.

Conclusions

Because of the central role of platelets in cardiovascular thrombosis, antiplatelet therapy (including the COX1 inhibitor aspirin, the $P2Y_{12}$ antagonist clopidogrel and integrin αIIbβ3 antagonists) is a well-established part of the treatment of cardiovascular arterial disease. However, there remains a considerable incidence of arterial thrombosis in patients receiving currently available antiplatelet therapy. A poor response to aspirin and/or clopidogrel as measured by *in vitro* platelet function assays is associated with a poor clinical response to these drugs. Although part of this association may be the result of patient non-compliance, the response to clopidogrel is dependent on factors that decrease the metabolism of clopidogrel by cytochrome P450 in the liver (such as the reduced-function *CYP2C19* allele) or increase this process (such as cigarette smoking). The clinical benefit of changing aspirin and/or clopidogrel treatment based on platelet function tests and/or single nucleotide polymorphisms is as yet unproven, but studies are in progress to address this issue.

Novel $P2Y_{12}$ antagonists (prasugrel, ticagrelor, cangrelor and elinogrel) have advantages over clopidogrel, including more rapid, less variable and more complete inhibition of platelet function. Ongoing studies will determine whether these new $P2Y_{12}$ antagonists will result in better and/or more rapid antithrombotic effects than clopidogrel, without an unacceptable increase in haemorrhagic or other side effects — as has been recently reported in some clinical settings for ticagrelor[79] and prasugrel[73,74]. Many other novel antiplatelet agents (for example, antagonists of PAR1, GPVI, integrin α2β1, $5HT_{2A}$ and novel integrin αIIbβ3 epitopes) are in development as antithrombotic agents.

An improved understanding of the mechanisms by which platelets become activated has been essential to the development of novel and improved antiplatelet therapies[137]. In nucleated cells, the study of signal transduction has relied on genetics and molecular biology (including deletion, overexpression and modification of specific gene products) to identify and map signalling pathways. However, in the anucleate platelet, genetic manipulation is more complicated, requiring the use of model cell lines or engineering of genetically modified animals[137]. Platelet biologists have therefore been more reliant on pharmacological manipulation using small molecules to investigate platelet signalling pathways. However, target-directed screening, high-throughput screening, chemical genetics, bioinformatics and proteomic approaches are increasingly being used[137,138].

A fundamental question is how to improve the prevention and treatment of platelet-dependent thrombosis without causing an increase in haemorrhagic side effects. An agent that can achieve this is the ultimate goal of drug discovery in the field of antiplatelet therapy for the treatment of cardiovascular disease.

1. Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ* **324**,71–86 (2002).
2. Michelson, A. D. (ed.) *Platelets* 2nd edn (Elsevier/Academic Press, San Diego, 2007).
3. Nurden, A. T., Nurden, P., Sanchez, M., Andia, I. & Anitua, E. Platelets and wound healing. *Front. Biosci.* **13**, 3532–3548 (2008).
4. Furman, M. I. *et al.* Release of soluble CD40L from platelets is regulated by glycoprotein IIb/IIIa and actin polymerization. *J. Am. Coll. Cardiol.* **43**, 2319–2325 (2004).
5. Trier, D. A. *et al.* Platelet antistaphylococcal responses occur through $P2X_1$ and $P2Y_{12}$ receptor-induced activation and kinocidin release. *Infect. Immun.* **76**, 5706–5713 (2008).
6. Italiano, J. E. Jr *et al.* Angiogenesis is regulated by a novel mechanism: pro- and antiangiogenic proteins are organized into separate platelet alpha granules and differentially released. *Blood* **111**, 1227–1233 (2008).
7. Nierodzik, M. L., Karpatkin, S. in *Platelets* 2nd edn (ed. Michelson, A. D.) 769–778 (Elsevier/Academic Press, San Diego, 2007).
8. White, J. G. in *Platelets* 2nd edn (ed. Michelson, A. D.) 45–73 (Elsevier/Academic Press, San Diego, 2007).
9. Italiano, J. E. & Hartwig, J. H. in *Platelets* 2nd edn (ed. Michelson, A. D.) 23–44 (Elsevier/Academic Press, San Diego, 2007).
10. Levin, J. in *Platelets* 2nd edn (ed. Michelson, A. D.) 3–22 (Elsevier/Academic Press, San Diego, 2007).
11. Clemetson, K. J. & Clemetson, J. M. in *Platelets* 2nd edn (ed. Michelson, A. D.) 117–143 (Elsevier/Academic Press, San Diego, 2007).
12. Hartwig, J. H. in *Platelets* 2nd edn (ed. Michelson, A. D.) 75–97 (Elsevier/Academic Press, San Diego, 2007).
13. Rex, S. & Freedman, J. E. in *Platelets* 2nd edn (ed. Michelson, A. D.) 251–279 (Elsevier/Academic Press, San Diego, 2007).
14. Brass, L. F., Stalker, T. J., Zhu, L. & Woulfe, D. S. in *Platelets* 2nd edn (ed. Michelson, A. D.) 319–346 (Elsevier/Academic Press, San Diego, 2007).
15. Roth, G. J., Stanford, N. & Majerus, P. W. Acetylation of prostaglandin synthase by aspirin. *Proc. Natl Acad. Sci. USA* **72**, 3073–3076 (1975).
16. Loll, P. J., Picot, D. & Garavito, R. M. The structural basis of aspirin activity inferred from the crystal structure of inactivated prostaglandin H2 synthase. *Nature Struct. Biol.* **2**, 637–643 (1995).
17. Patrono, C., Baigent, C., Hirsh, J. & Roth, G. Antiplatelet drugs. American College of Chest Physicians evidence-based clinical practice guidelines (8th edition). *Chest* **133**, S199–S233 (2008).
The most recent evidence-based guidelines for the use of current FDA-approved antiplatelet therapy.
18. Frelinger, A. L. *et al.* Lack of association between cyclooxygenase-1-dependent platelet function assays and adverse clinical outcomes in 700 consecutive aspirin-treated patients presenting for cardiac catheterization. *J. Am. Coll. Cardiol.* **51**, A370 (2008).
19. Michelson, A. D., Frelinger, A. L. & Furman, M. I. Resistance to antiplatelet drugs. *Eur. Heart J.* **8**, G53–G58 (2006).
20. Snoep, J. D., Hovens, M. M., Eikenboom, J. C., van der Bom, J. G. & Huisman, M. V. Association of laboratory-defined aspirin resistance with a higher risk of recurrent cardiovascular events: a systematic review and meta-analysis. *Arch. Intern. Med.* **167**, 1593–1599 (2007).
21. Krasopoulos, G., Brister, S. J., Beattie, W. S. & Buchanan, M. R. Aspirin "resistance" and risk of cardiovascular morbidity: systematic review and meta-analysis. *BMJ* **336**, 195–203 (2008).
22. Frelinger, A. L. *et al.* Aspirin 'resistance': role of pre-existent platelet reactivity and correlation between tests. *J. Thromb.Haemost.* **6**, 2035–2044 (2008).
23. Cattaneo, M. in *Platelets* 2nd edn (ed. Michelson, A. D.) 201–220 (Elsevier/Academic Press, San Diego, 2007).
24. Cattaneo, M. in *Platelets* 2nd edn (ed. Michelson, A. D.) 1127–1144 (Elsevier/Academic Press, San Diego, 2007).
25. Michelson, A. D. $P2Y_{12}$ antagonism: promises and challenges. *Arterioscler. Thromb. Vasc. Biol.* **28**, S33–S38 (2008).
26. Bertrand, M. E., Rupprecht, H. J., Urban, P. & Gershlick, A. H. Double-blind study of the safety of clopidogrel with and without a loading dose in combination with aspirin compared with ticlopidine in combination with aspirin after coronary stenting: the clopidogrel aspirin stent international cooperative study (CLASSICS). *Circulation* **102**, 624–629 (2000).
27. Yusuf, S. *et al.* Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N. Engl. J. Med.* **345**, 494–502 (2001).
28. Mehta, S. R. *et al.* Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. *Lancet* **358**, 527–533 (2001).
29. Steinhubl, S. R. *et al.* Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. *JAMA* **288**, 2411–2420 (2002).

30. Chen, Z. M. *et al.* Addition of clopidogrel to aspirin in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial. *Lancet* **366**, 1607–1621 (2005).
31. Sabatine, M. S. *et al.* Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation. *N. Engl. J. Med.* **352**, 1179–1189 (2005).
32. Bhatt, D. L. *et al.* Clopidogrel and aspirin versus aspirin alone for the prevention of atherothrombotic events. *N. Engl. J. Med.* **354**,1706–1717 (2006).
33. Wang, T. H. *et al.* An analysis of mortality rates with dual-antiplatelet therapy in the primary prevention population of the CHARISMA trial. *Eur. Heart J.* **28**, 2200–2207 (2007).
34. Snoep, J. D. *et al.* Clopidogrel nonresponsiveness in patients undergoing percutaneous coronary intervention with stenting: a systematic review and meta-analysis. *Am. Heart J.* **154**, 221–231 (2007).
35. Matetzky, S. *et al.* Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. *Circulation* **109**, 3171–3175 (2004).
36. Gurbel, P. A. *et al.* Clopidogrel effect on platelet reactivity in patients with stent thrombosis. Results of the CREST study. *J. Am. Coll. Cardiol.* **46**, 1827–1832 (2005).
37. Cuisset, T. *et al.* High post-treatment platelet reactivity identified low-responders to dual antiplatelet therapy at increased risk of recurrent cardiovascular events after stenting for acute coronary syndrome. *J. Thromb. Haemost.* **4**, 542–549 (2006).
38. Hochholzer, W. *et al.* Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. *J. Am. Coll. Cardiol.* **48**, 1742–1750 (2006).
39. Bliden, K. P. *et al.* Increased risk in patients with high platelet aggregation receiving chronic clopidogrel therapy undergoing percutaneous coronary intervention: is the current antiplatelet therapy adequate? *J. Am. Coll. Cardiol.* **49**, 657–666 (2007).
40. Buonamici, P. *et al.* Impact of platelet reactivity after clopidogrel administration on drug-eluting stent thrombosis. *J. Am. Coll. Cardiol.* **49**, 2312–2317 (2007).
41. Sibbing, D. *et al.* Platelet reactivity after clopidogrel treatment assessed with point-of-care analysis and early drug-eluting stent thrombosis. *J. Am. Coll. Cardiol.* **53**, 849–856 (2009).
Whole-blood platelet aggregation, measured by an automated impedance method, was used to show that low response to clopidogrel is significantly associated with an increased risk of drug-eluting stent thrombosis in patients with coronary artery disease.
42. Marcucci, R. *et al.* Cardiovascular death and nonfatal myocardial infarction in acute coronary syndrome patients receiving coronary stenting are predicted by residual platelet reactivity to ADP detected by a point-of-care assay: a 12-month follow-up. *Circulation* **119**, 237–242 (2009).
Residual platelet reactivity to ADP after clopidogrel therapy, measured by the point-of-care VerifyNow P2Y12 Assay, is able to detect ACS patients at risk of 12-month cardiovascular death and non-fatal myocardial infarction.
43. Bonello, L. *et al.* Vasodilator-stimulated phosphoprotein phosphorylation analysis prior to percutaneous coronary intervention for exclusion of postprocedural major adverse cardiovascular events. *J. Thromb. Haemost.* **5**, 1630–1636 (2007).
44. Bonello, L. *et al.* Adjusted clopidogrel loading doses according to vasodilator-stimulated phosphoprotein phosphorylation index decrease rate of major adverse cardiovascular events in patients with clopidogrel resistance. A multicenter randomized prospective study. *J. Am. Coll. Cardiol.* **51**, 1404–1411 (2008).
Although limited, this was the first study to suggest that adjusting the clopidogrel loading dose according to platelet monitoring using the VASP index is safe and may significantly improve the clinical outcome after PCI in patients with clopidogrel hyporesponsiveness despite a first 600-mg loading dose.
45. Angiolillo, D. J. *et al.* Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. *J. Am. Coll. Cardiol.* **49**, 1505–1516 (2007).
46. Roy, P. *et al.* Impact of "nuisance" bleeding on clopidogrel compliance in patients undergoing intracoronary drug-eluting stent implantation. *Am. J. Cardiol.* **102**, 1614–1617 (2008).
47. Sorensen, R. *et al.* Initiation and persistence with clopidogrel treatment after acute myocardial infarction: a nationwide study. *Brit. J. Clin. Pharmacol.* **66**, 875–884 (2008).
48. Gilard, M. *et al.* Influence of omeprazole on the antiplatelet action of clopidogrel associated with aspirin: the randomized, double-blind OCLA (Omeprazole CLopidogrel Aspirin) study. *J. Am. Coll. Cardiol.* **51**, 256–260 (2008).
49. Ho, P. M. *et al.* Risk of adverse outcomes associated with concomitant use of clopidogrel and proton pump inhibitors following acute coronary syndrome. *JAMA* **301**, 937–944 (2009).
50. Siller-Matula, J. M. *et al.* Effects of pantoprazole and esomeprazole on platelet inhibition by clopidogrel. *Am. Heart J.* **157**, 148.e1–148.e5 (2009).
51. Sibbing, D. *et al.* Impact of proton pump inhibitors on the antiplatelet effects of clopidogrel. *Thromb. Haemost.* **101**, 714–719 (2009).
52. O'Donoghue, M. L. *et al.* Pharmacodynamic and clinical efficacy of clopidogrel and of prasugrel with or without a proton-pump inhibitor: an analysis of two randomised trials. *Lancet* **374**, 989–997 (2009).
53. Bliden, K. P. *et al.* The association of cigarette smoking with enhanced platelet inhibition by clopidogrel. *J. Am. Coll. Cardiol.* **52**, 531–533 (2008).
As determined by platelet aggregometry and flow cytometry, clopidogrel therapy in current cigarette smokers was associated with increased inhibition of platelet function compared with non-smokers.
54. Desai, N. R., Mega, J. L., Jiang, S., Cannon, C. P. & Sabatine, M. S. Interaction between cigarette smoking and clinical benefit of clopidogrel. *J. Am. Coll. Cardiol.* **53**, 1273–1278 (2009).
This study provided evidence that cigarette smoking positively modifies the beneficial effect of clopidogrel on angiographic and clinical outcomes.
55. Mega, J. L. *et al.* Cytochrome p-450 polymorphisms and response to clopidogrel. *N. Engl. J. Med.* **360**, 354–362 (2009).
56. Simon, T. *et al.* Genetic determinants of response to clopidogrel and cardiovascular events. *N. Engl. J. Med.* **360**, 363–375 (2009).
57. Collet, J. P. *et al.* Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. *Lancet* **373**, 309–317 (2009).
References 55–57 showed that a common reduced-function *CYP2C19* allele results in significantly lower levels of the active metabolite of clopidogrel with resultant diminished platelet inhibition and a higher rate of major adverse cardiovascular events.
58. Michelson, A. D. *et al.* Evidence that pre-existent variability in platelet response to ADP accounts for 'clopidogrel resistance'. *J. Thromb. Haemost.* **5**, 75–81 (2007).
59. Angiolillo, D. J. *et al.* Identification of low responders to a 300-mg clopidogrel loading dose in patients undergoing coronary stenting. *Thromb. Res.* **115**, 101–108 (2005).
60. Angiolillo, D. J. *et al.* High clopidogrel loading dose during coronary stenting: effects on drug response and interindividual variability. *Eur. Heart J.* **25**, 1903–1910 (2004).
61. Samara, W. M., Bliden, K. P., Tantry, U. S. & Gurbel, P. A. The difference between clopidogrel responsiveness and posttreatment platelet reactivity. *Thromb. Res.* **115**, 89–94 (2005).
62. Gurbel, P. A., Bliden, K. P., Hiatt, B. L. & O'Connor, C. M. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. *Circulation* **107**, 2908–2913 (2003).
63. Angiolillo, D. J. *et al.* Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. *Diabetes* **54**, 2430–2435 (2005).
64. Holmes, D. R. Jr *et al.* Thrombosis and drug-eluting stents: an objective appraisal. *J. Am. Coll. Cardiol.* **50**,109–118 (2007).
65. Jakubowski, J. A., Winters, K. J., Naganuma, H. & Wallentin, L. Prasugrel: a novel thienopyridine antiplatelet agent. A review of preclinical and clinical studies and the mechanistic basis for its distinct antiplatelet profile. *Cardiovas Drug Rev.* **25**, 357–374 (2007).
66. Sugidachi, A. *et al.* The greater *in vivo* antiplatelet effects of prasugrel as compared to clopidogrel reflect more efficient generation of its active metabolite with similar antiplatelet activity to that of clopidogrel's active metabolite. *J. Thromb. Haemost.* **5**, 1545–1551(2007).
This study provides a mechanistic explanation for why prasugrel is a more potent $P2Y_{12}$ antagonist than clopidogrel.
67. Brandt, J. T. *et al.* A comparison of prasugrel and clopidogrel loading doses on platelet function: magnitude of platelet inhibition is related to active metabolite formation. *Am. Heart J.* **153**, 66 (2007).
68. Jernberg, T. *et al.* Prasugrel achieves greater inhibition of platelet aggregation and a lower rate of non-responders compared with clopidogrel in aspirin-treated patients with stable coronary artery disease. *Eur. Heart J.* **27**, 1166–1173 (2006).
69. Payne, C. D. *et al.* Increased active metabolite formation explains the greater platelet inhibition with prasugrel compared to high-dose clopidogrel. *J. Cardiovasc. Pharmacol.* **50**, 555–562 (2007).
70. Wiviott, S. D. *et al.* Prasugrel compared with high loading- and maintenance-dose clopidogrel in patients with planned percutaneous coronary intervention: the Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation-Thrombolysis in Myocardial Infarction 44 trial. *Circulation* **116**, 2923–2932 (2007).
71. Michelson, A. D. *et al.* Pharmacodynamic assessment of platelet inhibition by prasugrel versus clopidogrel in the TRITON-TIMI 38 trial. *Eur. Heart, J.* **30**, 1753–1763 (2009).
72. Wiviott, S. D. *et al.* Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N. Engl. J. Med.* **357**, 2001–2015 (2007).
The definitive Phase III clinical trial of prasugrel versus clopidogrel.
73. Montalescot, G. *et al.* Prasugrel compared with clopidogrel in patients undergoing percutaneous coronary intervention for ST-elevation myocardial infarction (TRITON-TIMI 38): double-blind, randomised controlled trial. *Lancet* **373**, 723–731 (2009).
In this prespecified TRITON-TIMI 38 study of 3,534 STEMI patients undergoing PCI, prasugrel was more effective than clopidogrel for prevention of ischaemic events, without an apparent excess in bleeding.
74. Wiviott, S. D. *et al.* Greater clinical benefit of more intensive oral antiplatelet therapy with prasugrel in patients with diabetes mellitus in the trial to assess improvement in therapeutic outcomes by optimizing platelet inhibition with prasugrel-Thrombolysis in Myocardial Infarction 38. *Circulation* **118**, 1626–1636 (2008).
75. van Giezen, J. J. & Humphries, R. G. Preclinical and clinical studies with selective reversible direct P2Y12 antagonists. *Semin. Thromb. Hemost.* **31**, 195–204 (2005).
76. Husted, S. *et al.* Pharmacodynamics, pharmacokinetics, and safety of the oral reversible P2Y12 antagonist AZD6140 with aspirin in patients with atherosclerosis: a double-blind comparison to clopidogrel with aspirin. *Eur. Heart J.* **27**,1038–1047 (2006).
77. Cannon, C. P. *et al.* Safety, tolerability, and initial efficacy of AZD6140, the first reversible oral adenosine diphosphate receptor antagonist, compared with clopidogrel, in patients with non-ST-segment elevation acute coronary syndrome: primary results of the DISPERSE-2 trial. *J. Am. Coll. Cardiol.* **50**,1844–1851 (2007); erratum **50**, 2196 (2007).
78. Storey, R. F. *et al.* Inhibition of platelet aggregation by AZD6140, a reversible oral P2Y12 receptor antagonist, compared with clopidogrel in patients with acute coronary syndromes. *J. Am. Coll. Cardiol.* **50**,1852–1856 (2007).
79. Wallentin, L. *et al.* Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N. Engl. J. Med.* **361**, 1045–1057 (2009).
The definitive Phase III clinical trial of ticagrelor versus clopidogrel.
80. Storey, R. F., Oldroyd, K. G. & Wilcox, R. G. Open multicentre study of the P2T receptor antagonist AR-C69931MX assessing safety, tolerability and activity in patients with acute coronary syndromes. *Thromb. Haemost.* **85**, 401–407 (2001).

81. Greenbaum, A. B. *et al.* Initial experience with an intravenous P2Y12 platelet receptor antagonist in patients undergoing percutaneous coronary intervention: results from a 2-part, phase II, multicenter, randomized, placebo- and active-controlled trial. *Am. Heart J.* **151**, 689 (2006).
82. Gretler, D. D. *et al.* "First in human" experience with PRT060128, a new direct-acting, reversible, $P2Y_{12}$ inhibitor for IV and oral use. *J. Am. Coll. Cardiol.* **49** (suppl. 2), 326A (2007).
83. Chang, H., *et al.* Modified diadenosine tetraphosphonate derivatives synergistically inhibit platelet activation via both $P2Y_1$ and $P2Y_{12}$. *J. Am. Coll. Cardiol.* **51**, A281 (2008).
84. Agah, R., Plow, E. F. & Topol, E. J. in *Platelets* 2nd edn (ed. Michelson, A. D.) 1145–1163 (Elsevier/Academic Press, San Diego, 2007).
85. Nurden, A. T. & Caen, J. P. An abnormal platelet glycoprotein pattern in three cases of Glanzmann's thrombasthenia. *Br. J. Haematol.* **28**, 253–260 (1974).
86. Coller, B. S. Platelet GPIIb/IIIa antagonists: the first anti-integrin receptor therapeutics. *J. Clin. Invest.* **99**, 1467–1471 (1997).
87. Ammar, T., Scudder, L. E. & Coller, B. S. *In vitro* effects of the platelet glycoprotein IIb/IIIa receptor antagonist c7E3 Fab on the activated clotting time. *Circulation* **95**, 614–617 (1997).
88. Reverter, J. C. *et al.* Inhibition of platelet-mediated, tissue factor-induced thrombin generation by the mouse/human chimeric 7E3 antibody. Potential implications for the effect of c7E3 Fab treatment on acute thrombosis and "clinical restenosis". *J. Clin. Invest.* **98**, 863–874 (1996).
89. Furman, M. I. *et al.* GPIIb-IIIa antagonist-induced reduction in platelet surface factor V/Va binding and phosphatidylserine expression in whole blood. *Thromb. Haemost.* **84**, 492–498 (2000).
90. Byzova, T. V. & Plow, E. F. Networking in the hemostatic system. Integrin alphaIIb beta3 binds prothrombin and influences its activation. *J. Biol. Chem.* **272**, 27183–27188 (1997).
91. Mukherjee, D. & Roffi, M. Glycoprotein IIb/IIIa receptor inhibitors in 2008: do they still have a role? *J. Interv. Cardiol.* **21**, 118–121 (2008).
92. Chew, D. P., Bhatt, D. L., Sapp, S. & Topol, E. J. Increased mortality with oral platelet glycoprotein IIb/IIIa antagonists: a meta-analysis of phase III multicenter randomized trials. *Circulation* **103**, 201–206 (2001).
93. Du, X. P. *et al.* Ligands "activate" integrin alpha IIb beta 3 (platelet GPIIb-IIIa). *Cell* **65**, 409–416 (1991).
94. Blue, R., Murcia, M., Karan, C., Jirouskova, M. & Coller, B. S. Application of high-throughput screening to identify a novel α-specific small-molecule inhibitor of αIIbβ3-mediated platelet interaction with fibrinogen. *Blood* **111**, 1248–1256 (2008).
A novel approach to the development of an antagonist of integrin αIIbβ3 with a diminished capacity to induce the possibly disadvantageous conformational changes in this target.
95. Escher, R. *et al.* Antiaggregatory and proangiogenic effects of a novel recombinant human dual specificity anti-integrin antibody. *J. Thromb. Haemost.* **7**, 460–469 (2009).
96. Stoll, P. *et al.* Targeting ligand-induced binding sites on GPIIb/IIIa via single-chain antibody allows effective anticoagulation without bleeding time prolongation. *Arterioscler. Thromb. Vasc. Biol.* **27**, 1206–1212 (2007).
97. Eisert, W. G. in *Platelets* 2nd edn (ed. Michelson, A. D.) 1165–1179 (Elsevier/Academic Press, San Diego, 2007).
98. Diener, H. C. *et al.* European Stroke Prevention Study. 2. Dipyridamole and acetylsalicylic acid in the secondary prevention of stroke. *J. Neurol. Sci.* **143**, 1–13 (1996).
99. ESPRIT Study Group *et al.* Aspirin plus dipyridamole versus aspirin alone after cerebral ischaemia of arterial origin (ESPRIT): randomised controlled trial. *Lancet* **367**, 1665–1673 (2006).
100. Sacco, R. L. *et al.* Aspirin and extended-release dipyridamole versus clopidogrel for recurrent stroke. *N. Engl. J. Med.* **359**, 1238–1251 (2008).
101. Ikeda, Y., Sudo, T. & Kimura, Y. in *Platelets* 2nd edn (ed. Michelson, A. D.) 1181–1191 (Elsevier/Academic Press, San Diego, 2007).
102. Biondi-Zoccai, G. G. *et al.* Systematic review and meta-analysis of randomized clinical trials appraising the impact of cilostazol after percutaneous coronary intervention. *Am. Heart J.* **155**,1081–1089 (2008).
103. Lee, S. W. *et al.* Comparison of triple versus dual antiplatelet therapy after drug-eluting stent implantation (from the DECLARE-Long trial). *Am. J. Cardiol.* **100**,1103–1108 (2007).
104. Angiolillo, D. J. *et al.* A randomized study assessing the impact of cilostazol on platelet function profiles in patients with diabetes mellitus and coronary artery disease on dual antiplatelet therapy: results of the OPTIMUS-2 study. *Eur. Heart J.* **29**, 2202–2211 (2008).
105. Bahou, W. F. in *Platelets* 2nd edn (ed. Michelson, A. D.) 179–200 (Elsevier/Academic Press, San Diego, 2007).
106. Becker, R. C. *et al.* Safety and tolerability of SCH 530348 in patients undergoing non-urgent percutaneous coronary intervention: a randomised, double-blind, placebo-controlled phase II study. *Lancet* **373**, 919–928 (2009).
A Phase II trial demonstrating that a PAR1 antagonist inhibited PAR1 thrombin receptor activating peptide (TRAP)-induced platelet aggregation in a dose-dependent manner, was generally well tolerated and did not cause an increase in major bleeding, even when administered with aspirin and clopidogrel.
107. Matsuoka, T. *et al.* Inhibitory effect of E5555, an orally active thrombin receptor antagonist, on intimal hyperplasia following balloon injury. *J. Am. Coll. Cardiol.* **43**, 68A (2004).
108. Hsu, C. C., Wu, W. B. & Huang, T. F. A snake venom metalloproteinase, kistomin, cleaves platelet glycoprotein VI and impairs platelet functions. *J. Thromb. Haemost.* **6**, 1578–1585 (2008).
109. Takayama, H. *et al.* A novel antiplatelet antibody therapy that induces cAMP-dependent endocytosis of the GPVI/Fc receptor gamma-chain complex. *J. Clin. Invest.* **118**, 1785–1795 (2008).
110. Miller, M. W. *et al.* Small-molecule inhibitors of integrin α2β1 that prevent pathological thrombus formation via an allosteric mechanism. *Proc. Natl Acad. Sci. USA* **106**, 719–724 (2009).
111. Przyklenk, K. *et al.* Targeted inhibition of the serotonin $5\text{-}HT_{2A}$ receptor improves coronary patency in an *in vivo* model of recurrent thrombosis. *Circulation* **116**, II_206 (2007).
112. Wallace, J. L. *et al. In vivo* antithrombotic effects of a nitric oxide-releasing aspirin derivative, NCX-4016. *Thromb. Res.* **93**, 43–50 (1999).
113. Lorusso, R. *et al.* Functional effects of nitric oxide-releasing aspirin on vein conduits of diabetic patients undergoing CABG. *Int. J. Cardiol.* **118**, 164–169 (2007).
114. Fontayne, A. *et al.* The humanized anti-glycoprotein Ib monoclonal antibody h6B4-Fab is a potent and safe antithrombotic in a high shear arterial thrombosis model in baboons. *Thromb. Haemost.* **100**, 670–677 (2008).
115. Falati, S. *et al.* Accumulation of tissue factor into developing thrombi *in vivo* is dependent upon microparticle P-selectin glycoprotein ligand 1 and platelet P-selectin. *J. Exp Med.* **197**, 1585–1598 (2003).
116. Kumar, A. *et al.* Recombinant soluble form of PSGL-1 accelerates thrombolysis and prevents reocclusion in a porcine model. *Circulation* **99**,1363–1369 (1999).
117. Bedard, P. W. *et al.* Characterization of the novel P-selectin inhibitor PSI-697 [2-(4-chlorobenzyl)-3-hydroxy-7,8, 9,10-tetrahydrobenzo[h] quinoline-4-carboxylic acid] *in vitro* and in rodent models of vascular inflammation and thrombosis. *J. Pharmacol. Exp. Ther.* **324**, 497–506 (2008).
118. Meier, T. R. *et al.* Prophylactic P-selectin inhibition with PSI-421 promotes resolution of venous thrombosis without anticoagulation. *Thromb. Haemost.* **99**, 343–351 (2008).
119. Gresele, P. *et al.* of proaggregatory and antiaggregatory prostaglandins in hemostasis. Studies with combined thromboxane synthase inhibition and thromboxane receptor antagonism. *J. Clin. Invest.* **80**,1435–1445 (1987).
120. Fiddler, G. I. & Lumley, P. Preliminary clinical studies with thromboxane synthase inhibitors and thromboxane receptor blockers. A review. *Circulation* **81** (Suppl. 1), 78 (1990).
121. Osende, J. I., Shimbo, D., Fuster, V., Dubar, M. & Badimon, J. J. Antithrombotic effects of S18886, a novel orally active thromboxane A2 receptor antagonist. *J. Thromb. Haemost.* **2**, 492–498 (2004).
122. Belhassen, L., Pelle, G., Dubois-Rande, J. L. & Adnot, S. Improved endothelial function by the thromboxane A2 receptor antagonist S18886 in patients with coronary artery disease treated with aspirin. *J. Am. Coll. Cardiol.* **41**, 1198–1204 (2003).
123. Gaussem, P. *et al.* The specific thromboxane receptor antagonist S18886: pharmacokinetic and pharmacodynamic studies. *J. Thromb. Haemost.* **3**, 1437–1445 (2005).
124. Yoshida, M. *et al.* Distinct effects of z-335, a new thromboxane A2 receptor antagonist, on rabbit platelets and aortic smooth muscle. *Pharmacology* **79**, 50–56 (2007).
125. Qiao, N. *et al.* The thromboxane receptor antagonist PBT-3, a hepoxilin stable analog, selectively antagonizes the TPα isoform in transfected COS-7 cells. *J. Pharmacol. Exp. Ther.* **307**, 1142–1147 (2003).
126. Tsao, P. S. & Lefer, A. M. Cardioprotective actions of the specific thromboxane receptor antagonist (+)-S145Na following coronary occlusion and reperfusion in the rat. *Res. Commun. Chem. Pathol. Pharmacol.* **70**, 205–211 (1990).
127. Ito, Y. *et al.* Effects of selective cyclooxygenase inhibitors on ischemia/reperfusion-induced hepatic microcirculatory dysfunction in mice. *Eur. Sur. Res.* **35**, 408–416 (2003).
128. Maassen, V. A. *et al*. Augmented contraction of the human isolated coronary artery by sumatriptan: a possible role for endogenous thromboxane. *Br. J. Pharmacol.* **119**, 855–862 (1996).
129. Brothers, T. E., Robison, J. G., Elliott, B. M., Boggs, J. M. & Halushka, P. V. Thromboxane A2 receptor density increases during chronic exposure to thromboxane A2 receptor antagonists after porcine carotid bypass. *Cardiovasc. Surg.* **5**, 92–98 (1997).
130. Neri Serneri, G. G., Coccheri, S., Marubini, E. & Violi, F. Drug Evaluation in Atherosclerotic Vascular Disease in Diabetics (DAVID) Study Group. Picotamide, a combined inhibitor of thromboxane A2 synthase and receptor, reduces 2-year mortality in diabetics with peripheral arterial disease: the DAVID study. *Eur. Heart J.* **25**,1845–1852 (2004).
131. Dogne, J. M. *et al.* Pharmacological evaluation of the novel thromboxane modulator BM-567 (I/II). Effects of BM-567 on platelet function. *Prostaglandins Leukot. Essent. Fatty Acids* **68**, 49–54 (2003).
132. Kolh, P. *et al.* Evaluation of BM-573, a novel TXA2 synthase inhibitor and receptor antagonist, in a porcine model of myocardial ischemia-reperfusion. *Prostaglandins Other Lipid Mediat.* **79**, 53–73 (2006).
133. Minuz, P. *et al.* Antiaggregating and vasodilatory effects of a new nitroderivative of acetylsalicylic acid. *Thromb. Res.* **80**, 367–376 (1995).
134. Singh, J. *et al.* Antagonists of the EP_3 receptor for prostaglandin E_2 are novel antiplatelet agents that do not prolong bleeding. *ACS Chem. Biol.* **4**, 115–126 (2009).
135. Yap, C. L. *et al.* Essential role for phosphoinositide 3-kinase in shear-dependent signaling between platelet glycoprotein Ib/V/IX and integrin αIIbβ3. *Blood* **99**,151–158 (2002).
136. Sturgeon, S. A., Jones, C., Angus, J. A. & Wright, C. E. Advantages of a selective beta-isoform phosphoinositide 3-kinase antagonist, an anti-thrombotic agent devoid of other cardiovascular actions in the rat. *Eur. J. Pharmacol.* **587**, 209–215 (2008).
137. Flaumenhaft, R. & Dilks, J. R. Discovery-based strategies for studying platelet function. *Mini Rev. Med. Chem.* **8**, 350–357 (2008).
138. Lewandrowski, U. *et al.* Platelet membrane proteomics: a novel repository for functional research. *Blood* **114**, e10–e19 (2009).
139. Michelson, A. D. Methods for the measurement of platelet function. *Am. J. Cardiol.* **103** (suppl. 3), 20A–6A (2009).
140. Steinhubl, S. R. in *Platelets* 2nd edn (ed. Michelson, A. D.) 509–518 (Elsevier/Academic Press, San Diego, 2007).
141. Michelson, A. D. *et al.* Platelet GP IIIa PI(A) polymorphisms display different sensitivities to agonists. *Circulation* **101**, 1013–1018 (2000).
142. Brandt, J. T. *et al.* Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. *J. Thromb. Haemost.* **5**, 2429–2436 (2007).

143. Mega, J. L. *et al.* Cytochrome P450 genetic polymorphisms and the response to prasugrel: relationship to pharmacokinetic, pharmacodynamic, and clinical outcomes. *Circulation* **119**, 2553–2560 (2009).
144. Bonello, L. *et al.* Tailored clopidogrel loading dose according to platelet reactivity monitoring to prevent acute and subacute stent thrombosis. *Am. J. Cardiol.* **103**, 5–10 (2009).
In this small study, tailoring the loading dose of clopidogrel according to platelet reactivity monitoring with the VASP assay decreased the rate of early stent thrombosis after PCI without increasing bleeding.
145. Cuisset, T. *et al.* Glycoprotein IIb/IIIa inhibitors improve outcome after coronary stenting in clopidogrel nonresponders. *J. Am. Coll. Cardiol. Cardiovasc. Inter.* **1**, 649–653 (2008).
146. Valgimigli, M. *et al.* Intensifying platelet inhibition with tirofiban in poor responders to aspirin, clopidogrel, or both agents undergoing elective coronary intervention. Results from the double-blind, prospective, randomized tailoring treatment with tirofiban in patients showing resistance to aspirin and/or resistance to clopidogrel study. *Circulation* **119**, 3215–3222 (2009).
147. Andrews, R. K., Berndt, M. C. & Lopez, J. A. in *Platelets* 2nd edn (ed. Michelson, A. D.) 145–163 (Elsevier/Academic Press, San Diego, 2007).
148. Plow, E. F., Pesho, M. M. & Ma, Y. Q. in *Platelets* 2nd edn (ed. Michelson, A. D.) 165–178 (Elsevier/Academic Press, San Diego, 2007).
149. McEver, R. P. in *Platelets* 2nd edn (ed. Michelson, A. D.) 231–249 (Elsevier/Academic Press, San Diego, 2007).
150. Hoffman, M. & Monroe, D. M. Coagulation 2006: a modern view of hemostasis. *Hematol. Oncol. Clin. N. Am.* **21**, 1–11 (2007).
151. Furie, B. & Furie, B. C. Mechanisms of thrombus formation. *N. Engl. J. Med.* **359**, 938–949 (2008).
152. Lordkipanidze, M. *et al.* A comparison of six major platelet function tests to determine the prevalence of aspirin resistance in patients with stable coronary artery disease. *Eur. Heart J.* **28**,1702–1708 (2007).

Competing interests statement

The author declares competing financial interests: see web version for details.

DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/gene
OMIM: http://www.ncbi.nlm.nih.gov/omim
UniProtKB: http://www.uniprot.org
COX1 | EP_3 | GP1bα | GPIIb | GPIIIa | GPVI | PAR1 | P-selectin | $P2Y_1$ | $P2Y_{12}$ | VWF

FURTHER INFORMATION

Alan D. Michelson's homepage: http://www.platelets.org/director.htm
BRIDGE: http://www.clinicaltrials.gov/ct2/show/NCT00767507
CHAMPION-PCI: www.clinicaltrials.gov/ct2/show/NCT00305162
CHAMPION-PLATFORM: http://www.clinicaltrials.gov/ct2/show/NCT00385138
A double-blind study of E5555 in Japanese patients with acute coronary syndrome: http://www.clinicaltrials.gov/ct2/show/NCT00619164
DANTE trial: http://www.clinicaltrials.gov/ct2/show/NCT00774475
GRAVITAS: http://www.clinicaltrials.gov/ct2/show/NCT00645918
Medicines Company news release: http://ir.themedicinescompany.com/phoenix.zhtml?c=122204&p=irol-newsArticle&ID=1287788&highlight=
Safety and tolerability of E5555 and its effects on markers of intravascular inflammation in subjects with coronary artery disease: http://www.clinicaltrials.gov/ct2/show/NCT00312052
Safety and tolerability of E5555 and its effects on markers of intravascular inflammation in subjects with acute coronary syndrome: http://www.clinicaltrials.gov/ct2/show/NCT00548587
A study to determine the pharmacokinetics of elinogrel in healthy volunteers and patients with mild, moderate and severe renal impairment: http://www.clinicaltrials.gov/ct2/show/NCT00984113
3T/2R: www.clinicaltrials.gov/ct2/show/NCT00398463
TRACER: http://www.clinicaltrials.gov/ct2/show/NCT00527943
TRA 2P-TIMI 50: http://www.clinicaltrials.gov/ct2/show/NCT00526474
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