Novel Antiplatelet Therapies for Atherothrombotic Diseases

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Abstract—Antiplatelet therapies are an essential tool to reduce the risk of developing clinically apparent atherothrombotic disease and are a mainstay in the therapy of patients who have established cardiovascular, cerebrovascular, and peripheral artery disease. Strategies to intensify antiplatelet regimens are limited by concomitant increases in clinically significant bleeding. The development of novel antiplatelet therapies targeting additional receptor and signaling pathways, with a focus on maintaining antiplatelet efficacy while preserving hemostasis, holds tremendous potential to improve outcomes among patients with atherothrombotic diseases.

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Key Words: acute coronary syndrome ■ cardiovascular disease ■ hemostasis ■ myocardial infarction ■ thrombosis

Atherosclerosis is a pan-vascular arterial disease process involving the coronary, cerebral, and peripheral arteries and remains the leading cause of mortality in the urbanized areas.[1] The common pathophysiologic pathway of atherosclerosis ends in narrowing or obliteration of the arterial lumen through erosion or rupture of lipid-laden and highly inflammatory plaques, with subsequent thrombosis. The clinical manifestations correspond directly to the organ system affected, although atherosclerosis in 1 vascular bed is predictive of disease in other territories. Antiplatelet therapy remains a cornerstone in the management of patients with atherothrombotic diseases. The use of single or dual antiplatelet therapy (DAPT) regimens has been effective in reducing cardiovascular events among patients with stable coronary artery disease (CAD), acute coronary syndrome (ACS), peripheral artery disease (PAD), and cerebrovascular disease.

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During the past several years, oral and intravenous antiplatelet therapies have been developed with escalating potency to reduce further clinical atherothrombotic events among at-risk patients (Table; Figure 1). However, adoption of these agents has occurred with a concomitant increase in clinically significant bleeding. Consequently, there has been an interest in additional strategies to improve net clinical outcomes, such as the development of tools to predict individual bleeding and ischemic risk, minimizing antiplatelet exposure among patients with low ischemic or high bleeding risk, and improving percutaneous stent technologies to mitigate late thrombotic risks. Additionally, there are now focused and innovative efforts to develop novel pharmacotherapies which target receptors and pathways in the thrombotic process while preserving the normal hemostatic function of platelets. Here, we review current state-of-the-art and novel antiplatelet strategies to treat atherothrombotic diseases.

Established Antiplatelet Therapies

Aspirin

Aspirin nonselectively and irreversibly acetylates a serine residue on the COX (cyclooxygenase) enzymes, suppressing the production of prostaglandins and TxA2 (thromboxane A2), a potent platelet activator. Aspirin is a foundation in antiplatelet regimens, both as a single agent, and in combination with other antiplatelet or antithrombotic agents, particularly for the secondary prevention of cardiovascular events. The landmark Antithrombotic Trialists' Collaboration meta-analysis of 287 studies including 212000 patients demonstrated the efficacy of aspirin in reducing nonfatal myocardial infarction (MI), stroke, and cardiovascular death among patients with ACS (new or old), stroke, or who were at increased risk for vascular events.[2] Based on this evidence, aspirin is commonly used for secondary prevention in patients with CAD, cerebrovascular accident, and PAD.

The role of aspirin for primary prevention of cardiovascular disease remains controversial and a topic of ongoing clinical investigation. A recent study randomized 19114 patients in Australia and the United States who were ≥70 years of age (or ≥65 years among blacks and Hispanics in the

Nonstandard Abbreviations and Acronyms	
12-LOX	12-lipoxygenase
ACS	acute coronary syndrome
CAD	coronary artery disease
CHAMPION	Clopidogrel Versus Standard Therapy to Achieve Optimal Management of Platelet Inhibition
CHANCE	Clopidogrel in High-Risk Patients With Acute Nondisabling Cerebrovascular Events
CHARISMA	Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance
CVD	cerebrovascular disease
DAPT	dual antiplatelet therapy
EUCLID	Examining Use of Ticagrelor in Peripheral Artery Disease
GP	glycoprotein
MI	myocardial infarction
PAD	peripheral artery disease
PAR	protease-activated receptor
PCI	percutaneous coronary intervention
PDI	protein disulfide-isomerase
PEGASUS-TIMI 54	Prevention of Cardiovascular Events in Patients With Prior Heart Attack Using Ticagrelor Compared to Placebo on a Background of Aspirin–Thrombolysis in Myocardial Infarction 54
PI3KB	phosphatidylinositol 3 kinase B
PLATO	Study of Platelet Inhibition and Patient Outcomes
POINT	Platelet-Oriented Inhibition in New TIA and Minor Ischemic Stroke
scFv	single-chain variable fragments
SOCRATES	Acute Stroke or Transient Ischaemic Attack Treated With Aspirin or Ticagrelor and Patient Outcomes
THALES	Acute Stroke or Transient Ischaemic Attack Treated With Ticagrelor and Aspirin for Prevention of Stroke or Death
TIA	transient ischemic attack
TICO	ticagrelor Monotherapy After 3 Months in the Patients Treated With New Generation Sirolimus Stent for Acute Coronary Syndrome
TRA 2P TIMI 50	Thrombin Receptor Antagonist in Secondary Prevention of Atherothrombotic Ischemic Events–Thrombolysis in Myocardial Infraction 50
TRILOGY ACS	Targeted Platelet Inhibition to Clarify the Optimal Strategy to Medically Manage Acute Coronary Syndromes)
TRITON-TIMI 38	Trial of Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel-Thrombolysis in Myocardial Infarction 38
TWILIGHT	Ticagrelor with Aspirin or Along in High-Risk Patients After Coronary Intervention
TxA2	thromboxane A2
vWF	von Willebrand Factor

United States) without cardiovascular disease to receive 100 mg of enteric-coated aspirin or placebo.[3] After a median of 4.7 years of follow-up, there was no improvement in the rates of cardiovascular disease between groups but a significantly higher risk of hemorrhage among those randomized to aspirin.

A separate study randomized 15 480 patients with diabetes mellitus but without clinically apparent cardiovascular disease to receive enteric coated aspirin at a dose of 100 mg daily or placebo.[4] After a mean follow-up of 7.4 years, there was a 12% reduction in serious vascular events, although a 29% increase in major bleeding rates among aspirin-treated patients. Finally, a recent study of 12 546 patients across 7 countries with a moderate estimated risk of first cardiovascular event randomized to enteric-coated aspirin 100 mg daily or placebo followed for a median of 60 months found no difference in

rates of cardiovascular events but a >2-fold increase in gastrointestinal bleeding events.[5] Thus, the potential role of aspirin in primary prevention seems confined to high-risk primary prevention (based on older studies) or patients with diabetes mellitus at low bleeding risk but at high ischemic risk.

P2Y12 Receptor Antagonists

The P2Y12 receptor is a G-protein coupled receptor which binds ADP, stored in platelet dense granules until platelet activation. ADP binding to the P2Y12 receptor inhibits adenylate cyclase–mediated signaling and the formation of cyclic AMP, which consequently enhances sustained platelet aggregation through intracellular signal activation and conformational changes of the GP (glycoprotein) IIb/IIIa receptor. These conformational changes in the GP IIb/IIIa receptor augment its affinity for its major ligand, soluble fibrinogen. This sequence may be interrupted by various P2Y12 receptor antagonists.

Clopidogrel

Clopidogrel is an oral, irreversible, competitive, thienopyridine P2Y12 receptor antagonist, which has been widely studied and shown to reduce cardiovascular events among patients with atherosclerotic disease. Among high-risk patients with ischemic stroke, MI, or established PAD, clopidogrel monotherapy was associated with a 7.9% relative risk reduction in MI, ischemic stroke, vascular death, or rehospitalization compared with aspirin.[6] Among patients with ACS, the addition of clopidogrel to aspirin reduces ischemic events by 20% in the first 30 days and results in similar reductions between 30 days and 12 months of treatment.[7] Additionally, a subgroup analysis of the CHARISMA trial (Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance) suggested that patients with a history of MI, ischemic stroke, or symptomatic PAD, may benefit from escalation on antiplatelet therapy with the addition of clopidogrel to aspirin for the reduction for cardiovascular death, MI, or stroke.[8,9] Current practice patterns suggest a high utilization of DAPT even beyond 12 months, particularly among patients with CAD. Among patients with ACS in Europe and Asia, a recent study demonstrated that 57% of patients were receiving DAPT beyond 12 months.[10] In another registry cohort of patients from the United States, France, Germany, Italy, and Greece, 43% of patients with ACS and 57% of patients who underwent elective percutaneous coronary intervention (PCI) received DAPT at the end of 2 years of follow-up.[11]

The role of clopidogrel in the treatment of stroke and transient ischemic attack (TIA) has been recently investigated. The CHANCE trial (Clopidogrel in High-Risk Patients With Acute Nondisabling Cerebrovascular Events) was a randomized, double-blind, placebo-controlled trial that randomly assigned patients with small ischemic strokes or high-risk TIA to a combination of clopidogrel and aspirin or aspirin alone for 90 days.[12] The study, conducted in a Chinese population, demonstrated a reduction in stroke with combination therapy (8.2% versus 11.7%; hazard ratio [HR], 0.68; 95% CI, 0.57–0.81; p<0.001), without an increased risk of moderate or severe hemorrhage. Recently, the POINT trial (Platelet-Oriented Inhibition in New TIA and Minor Ischemic Stroke) tested this

Table.   Commonly Used and Approved Antiplatelet Therapies for Cardiovascular Diseases

	Drug Class	Mechanism	Half-Life	Duration of Action	Administration and Frequency	Indications
Aspirin	Nonsteroidal anti-inflammatory drug	Irreversible acetylation and inhibition of COX enzyme	Dose dependent	10 d	Oral, once daily	Secondary prevention of CVD, ACS±PCI, revascularization procedures
Clopidogrel	Thienopyridine, P2Y12 receptor antagonist	Competitive, irreversible, P2Y12 receptor blockade	6 h	5–7 d	Oral, once daily	ACS±PCI, PCI (elective), symptomatic, high risk, CVD
Prasugrel	Thienopyridine, P2Y12 receptor antagonist	Competitive, irreversible, P2Y12 receptor blockade	7 h	7–10 d	Oral, once daily	ACS+PCI only
Ticagrelor	Triazolopyrimidine, P2Y12 receptor antagonist	Noncompetitive, reversible, P2Y12 receptor blockade	8–12 h	3–5 d	Oral, twice daily	ACS±PCI, long term with history of ACS
Abciximab	Glycoprotein IIb/IIIa inhibitor	Fab antibody fragment that binds to IIb/IIIa receptor with high affinity and low dissociation	4 h	Platelet function restored 24–48 h after discontinuation	Intravenous	ACS+PCI, PCI only
Eptifibatide	Glycoprotein IIb/IIIa inhibitor	Competitive, reversible IIb/IIIa receptor blockade	2.5 h	Platelet function restored 4–8 h after discontinuation	Intravenous	ACS±PCI
Tirofiban	Glycoprotein IIb/IIIa inhibitor	Competitive, reversible IIb/IIIa receptor blockade	2 h	Platelet function restored 4–8 h after discontinuation	Intravenous	ACS±PCI, PCI only
Cangrelor	Nonthienopyridine, ATP analogue	Noncompetitive, reversible, P2Y12 receptor blockade	3–5 min	30–60 min	Intravenous	PCI (adjunctive)
Vorapaxar	PAR-1 antagonist	Reversible, PAR-1 receptor blockade	5–13 d	Inhibition of TRAP-induced platelet aggregation at a level of 50% after 4 wk discontinuation	Oral, once daily	History of MI or PAD

ACS indicates acute coronary syndrome; COX, cyclooxygenase; CVD, cardiovascular disease; MI, myocardial infarction; PAD, peripheral artery disease; PAR, protease activating receptor; PCI, percutaneous coronary intervention; and TRAP, thrombin receptor agonist peptide.

hypothesis in an international population and confirmed a reduction in major ischemic strokes (5.0% versus 6.5%; HR, 0.75; 95% CI, 0.59–0.95; p=0.02), but demonstrated an increase in major hemorrhage with combination therapy (0.9% versus 0.4%; HR, 2.32; 95% CI, 1.10–4.87; p=0.02).[13]

Recent investigations have sought to determine whether a pharmacogenetic-guided strategy to the use of clopidogrel as anti-platelet therapy may be beneficial. Clopidogrel is a prodrug that is extensively metabolized by the liver, mediated by the cytochrome P450 system. Clopidogrel is first metabolized to the 2-oxo-clopidogrel intermediate metabolite, with subsequent metabolism yielding a thiol derivative which is the active metabolite. This pathway is mediated by CYP3A4, CYP2C19, CYPA12, and CYP2B6.[14] Ex vivo platelet aggregation studies have demonstrated differences in antiplatelet effects of clopidogrel according to the CYP2C19 genotype. Specifically, the CYP2C19*1 allele yields fully functional metabolism, whereas the CYP2C19*2 and CYP2C19*3 alleles yield reduced metabolism and antiplatelet therapy. The association between CYP2C19 genotype and clinical outcomes among patients receiving clopidogrel therapy is not well established. A recent multisite investigation of 1815 patients sought to determine whether genotype-guided antiplatelet therapy improved outcomes among

patients undergoing PCI.[15] In this study, 31.5% of patients had a loss of function allele. Among those with a loss of function allele who received clopidogrel versus alternative antiplatelet therapy, there was a 2-fold risk of cardiovascular events, whereas there was no significant difference in major adverse cardiovascular events rates among those with a loss of function allele treated with an alternate antiplatelet agent and those without a loss of function allele treated with clopidogrel. This strategy has yet to be established as beneficial in a randomized trial setting.[16,17]

Overall, the primary role of clopidogrel in CAD remains as an adjunct to aspirin in DAPT regimens after PCI and for treatment of ACS with or without PCI.[18] The addition of clopidogrel to aspirin may be reasonable for treatment of patients with a small stroke or TIA within 24 hours for 21 days and for patients with recent stroke or TIA within 30 days attributable to severe stenosis of a major intracranial artery for a duration of 90 days.[19,20] Additionally, clopidogrel in addition to aspirin may be reasonable in select patients with symptomatic PAD after lower extremity revascularization to prevent limb-related events.[21,22]

Prasugrel

Prasugrel is an oral, irreversible, competitive, thienopyridine P2Y12 receptor antagonist. Prasugrel is approved
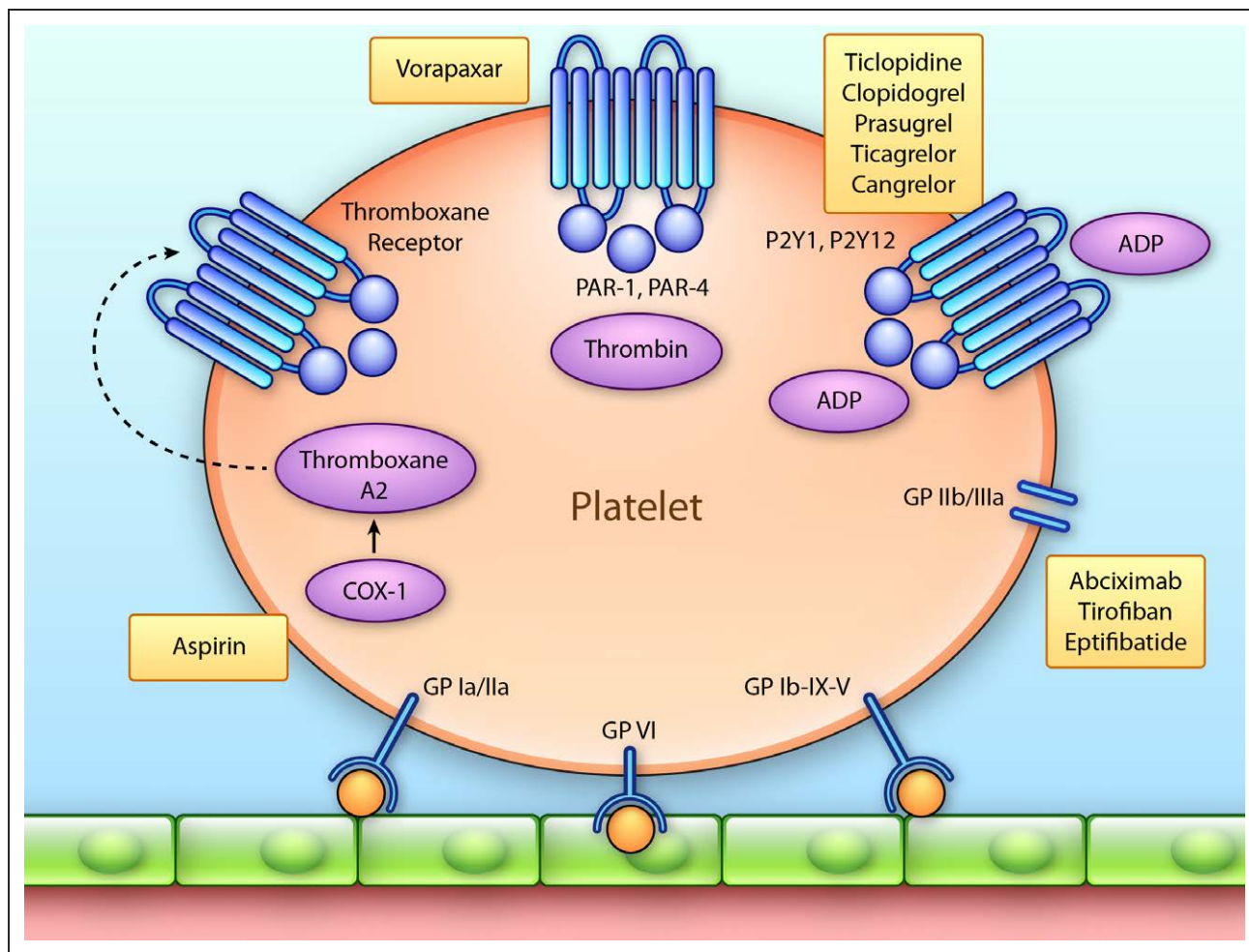
Figure 1. Commonly used and approved antiplatelet drugs and their targets. Platelet activation and aggregation occur through a complex interplay involving several platelet receptors and their ligands. Platelet adhesion initially occurs through interactions between GP (glycoprotein) Ib and von Willebrand factor, and GP VI and subendothelial collagen. Platelet activation additionally occurs through interactions of soluble agonists, such as TXA2 (thromboxane A2), and ADP which binds the P2Y12 receptor, promoting platelet aggregation. Intracellular signaling leads to conformation changes and activation of the GP IIb/IIIa receptor, enhancing its affinity for its major ligand, fibrinogen, which allows linking of platelets. The drugs depicted interrupt these pathways to provide antiplatelet activity. COX indicates cyclooxygenase; and PAR, protease activating receptor.

only for patients with CAD who present with ACS and undergo PCI, based on results of the TRITON-TIMI 38 study (Trial of Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel-Thrombolysis in Myocardial Infarction 38).[23] In this randomized study of 13 608 patients, prasugrel in addition to aspirin, lead to a 19% risk reduction of cardiovascular (CV) death, MI, or stroke compared with the combination of clopidogrel and aspirin. Subgroup analyses additionally revealed a lack of net clinical benefit and higher rates of major bleeding among patients age ≥75 years and with body weight <60 kg. Prasugrel is also contraindicated in patients with prior TIA or stroke.

The TRITON-TIMI 38 trial did not assess the role of prasugrel in medical management of ACS. However, the TRILOGY ACS study (Targeted Platelet Inhibition to Clarify the Optimal Strategy to Medically Manage Acute Coronary Syndromes) did not demonstrate improvement in the composite outcome of death, MI, or stroke among either patients under the age of 75 years or the entire study population when treated with prasugrel compared with clopidogrel for the

medical management of ACS.[24,25] However, there was a significant benefit with prasugrel over clopidogrel in TRILOGY ACS in those patients who had undergone coronary angiography, perhaps representing those patients who had true ACS versus troponin elevation because of other causes.[24]

Current guidelines suggest that prasugrel in place of clopidogrel is a reasonable option for antiplatelet therapy in addition to aspirin for patients with ACS treated with PCI.

Ticagrelor
Ticagrelor is an oral, direct acting, noncompetitive, reversible cyclopentyl triazolopyrimidine P2Y12 receptor antagonist. Compared with the thienopyridines, ticagrelor does not require hepatic metabolism for activation, is more rapidly acting, and more potent.

The landmark PLATO (Study of Platelet Inhibition and Patient Outcomes) randomized 18 624 patients presenting with ACS to ticagrelor or clopidogrel in addition to aspirin.[26] The study demonstrated a 16% risk reduction for the composite end point of CV mortality, MI, or stroke (9.8% versus

11.7%; HR, 0.84; 95% CI, 0.77–0.92; p<0.001), as well as a significant reduction in all-cause mortality (4.5% versus 5.9%; p<0.001) with ticagrelor. Ticagrelor was associated with higher rates of major noncoronary artery bypass grafting related bleeding and higher rates of intracranial hemorrhage.

A strategy of long term DAPT with ticagrelor and aspirin was evaluated among patients with a history of MI in the PEGASUS-TIMI 54 study (Prevention of Cardiovascular Events in Patients With Prior Heart Attack Using Ticagrelor Compared to Placebo on a Background of Aspirin–Thrombolysis in Myocardial Infarction 54).[27] This study randomized 21 162 patients with a history of MI 1 to 3 years before enrollment to receive ticagrelor 90 mg twice daily, ticagrelor 60 mg twice daily, or placebo, in addition to low-dose aspirin. After 33 months of follow-up, there was a 16% risk reduction in the composite outcome of CV death, MI, or stroke in the group that received 60 mg of ticagrelor twice daily (7.77% versus 9.04%; HR, 0.84; 95% CI, 0.74–0.95; p=0.004), with an increase in major bleeding compared with the placebo-controlled study arm. A PEGASUS-TIMI 54 substudy of patients with PAD demonstrated a 4.1% absolute reduction in major adverse cardiovascular events and a 35% relative risk reduction in major adverse limb events.[28] The subgroup with diabetes mellitus also showed significant relative and absolute benefits with ticagrelor, including lower CV mortality.[29]

The role of ticagrelor monotherapy among patients with PAD was assessed in the EUCLID study (Examining Use of Ticagrelor in Peripheral Artery Disease), which did not demonstrate a significant improvement in the composite primary outcome of CV death, MI, or ischemic stroke compared with clopidogrel.[30] Ticagrelor monotherapy for nonsevere ischemic stroke or high-risk TIA was assessed in the international SOCRATES study (Acute Stroke or Transient Ischaemic Attack Treated With Aspirin or Ticagrelor and Patient Outcomes) and did not demonstrate a significant reduction in time to occurrence of stroke, MI, or death within 90 days compared with aspirin.[31] Combination therapy with ticagrelor and aspirin for acute ischemic stroke or TIA is being evaluated in the THALES trial (Acute Stroke or Transient Ischaemic Attack Treated With Ticagrelor and Aspirin for Prevention of Stroke or Death; http://www.clinicaltrials.gov. Unique identifier: NCT03354429). The role of ticagrelor monotherapy compared with aspirin after PCI with a drug-eluting stent is being evaluated in the TWILIGHT (Ticagrelor with Aspirin or Along in High-Risk Patients After Coronary Intervention) and TICO (Ticagrelor Monotherapy After 3 Months in the Patients Treated With New Generation Sirolimus Stent for Acute Coronary Syndrome; http://www.clinicaltrials.gov. Unique identifier: NCT02494895) studies.[32]

There has additionally been interest in leveraging potential off-target effects of ticagrelor and other antiplatelet agents. However, a recent study of the effects of ticagrelor, prasugrel, and clopidogrel on endothelial function and vascular biomarkers found no difference in the reactive hyperemia index or biomarker levels in a population of post-ACS patients treated with the various agents.[33] Additionally, the study found no evidence that ticagrelor increases plasma adenosine levels compared with other antiplatelet agents, although this has been previously implicated as a mechanism to explain ticagrelor related side effects, including bradycardia and dyspnea.

Overall, current evidence supports ticagrelor as a first line agent for ACS with or without PCI in addition to aspirin, and for long term therapy among patients with a history of MI, assuming they are at low bleeding risk. The role of ticagrelor as an adjunct to aspirin in patients with stroke or PAD is uncertain, and the role of ticagrelor monotherapy in patients with CAD remains under investigation.

Cangrelor

Cangrelor is an intravenous, reversible, nonthienopyridine, ATP analog, P2Y12 receptor antagonist. Cangrelor has a rapid onset of action, with demonstrable platelet inhibition within 2 minutes, rapid reversibility with an elimination half-life of 3 to 6 minutes, and return of normal platelet function in 1 hour. Cangrelor was initially studied in the CHAMPION (Clopidogrel Versus Standard Therapy to Achieve Optimal Management of Platelet Inhibition) PCI and CHAMPION PLATFORM studies which suggested the possibility of a reduction in stent thrombosis without an excess of severe bleeding in patients treated with cangrelor.[34,35] Subsequently, the pivotal CHAMPION PHOENIX study prospectively evaluated the efficacy and safety of cangrelor versus clopidogrel pretreatment among patients undergoing urgent or elective PCI.[36] The primary efficacy end point, a composite of all-cause mortality, MI, ischemia-driven revascularization, or stent thrombosis 48 hours after randomization was significantly reduced in the cangrelor-treated study arm (4.7% versus 5.9%; adjusted odds ratio, 0.78; 95% CI, 0.66–0.93; p=0.005), with no significant difference in the primary safety end point. A pooled analysis of patient-level data from the 3 trials of cangrelor additionally supported this finding and demonstrated that cangrelor was associated with a 19% reduction in odds for the composite outcome of death, MI, ischemia-driven revascularization, or stent thrombosis at 48 hours.[37] Cangrelor use was also independently associated with a 38% reduction in odds for stent thrombosis (0.8% versus 1.4%; odds ratio, 0.62; 95% CI, 0.43–0.90; p=0.01).

Real-world experience with cangrelor has supported a potential role among patients with cardiogenic shock, who are uniquely at risk for gut malabsorption and likely to benefit from direct acting, intravenous agents.[38] However, these benefits must be balanced with the increased risk of bleeding among critically ill patients. Strategies to avoid potentiating bleeding risk in patients with and without cardiogenic shock, such as avoiding the concomitant use of glycoprotein IIb/IIIa inhibitors, are critical.[39] Presently, no randomized clinical trial evidence among patients with cardiogenic shock is available. Cangrelor had additionally been used off-label as a bridging therapy before cardiac surgery after discontinuation of thienopyridines, offering the potential advantage of rapid reversal of antiplatelet action with discontinuation 1 hour before surgery.[40] Additional evidence suggests that the benefits of cangrelor are maintained across several high-risk subgroups, including those with a history of stroke ≥1 year from the time of PCI, older patients, and in those on a background of anticoagulant therapy with heparin, without concomitant increases in the rates of major bleeding.[41–44]

PAR-1 Antagonists

The PAR (protease-activated receptor)-1 is the site of action of thrombin, which is highly active at sites of clotting. Vorapaxar is a competitive PAR-1 antagonist, which was studied among patients with a history of atherothrombotic events (MI, PAD, or stroke) in the TRA 2P TIMI 50 study (Thrombin Receptor Antagonist in Secondary Prevention of Atherothrombotic Ischemic Events– Thrombolysis in Myocardial Infraction 50).[45] The majority of patients enrolled in the study were on background antiplatelet therapy with aspirin, and a significant proportion was additionally being treated with a thienopyridine or dipyramidole. The trial was stopped early in patients with a history of stroke because of evidence of increased rates of intracranial hemorrhage. However, compared with patients receiving placebo, there was a reduction in the composite end point of cardiovascular death, MI, or stroke at 3 years with vorapaxar (9.3% versus 10.5%; HR, 0.87; 95% CI, 0.80–0.94; P<0.001), primarily driven by a reduction in MI. This came at the expense of increased moderate or severe bleeding (4.2% versus 2.5%; HR, 1.66; 95% CI, 1.43–1.93; P<0.001) and an increase in the rate of intracranial hemorrhage in the vorapaxar group (1.0% versus 0.5% in the placebo group; P<0.001). Vorapaxar is contraindicated in patients with a history of stroke, TIA, or intracerebral hemorrhage.[46]

In a substudy of patients with symptomatic lower extremity PAD, vorapaxar was associated with a reduction in acute limb ischemia (2.3% versus 3.9%; HR, 0.58; 95% CI, 0.39–0.86; p=0.006) and reduction in peripheral artery revascularization (18.4% versus 22.2%; HR, 0.84; 95% CI, 0.73–0.97; p=0.017).[47] However, in this cohort, bleeding occurred more frequently with vorapaxar (7.4% versus 4.5%; HR, 1.62; 95% CI, 1.21–2.18; p=0.001), and there was no reduction in the composite primary end point of cardiovascular death, MI, or stroke.

Experimental Therapies

Current approaches to improving antiplatelet efficacy have relied on pharmacotherapies with increasing potency. However, in virtually all cases, increasing potency is linked to a concomitant increase in bleeding. This observation has led to a paradigm shift as new targets, and novel approaches for antiplatelet therapies are being developed. Instead of simply developing more potent therapies, preclinical and early clinical studies have supported the notion that thrombosis pathways may be targeted while preserving hemostasis and may, therefore, maintain efficacy while improving safety compared with currently available therapies (Figure 2).

Developing thrombi are now known to consist of 2 distinct regions. The platelet response at the site of arterial injury comprises a core of fully activated platelets located close to the lesion and is highly dependent on soluble agonists such as thrombin. Separately, a propagating thrombus is composed primarily of platelets in lower activation states, and their recruitment is minimally sensitive to standard antiplatelet drugs.[48] This distinction between the hemostatic response, which relies on the thrombus core, and the thrombotic response that regulates the growth of a propagating outer shell of thrombus has provided a conceptual framework for developing novel therapies.
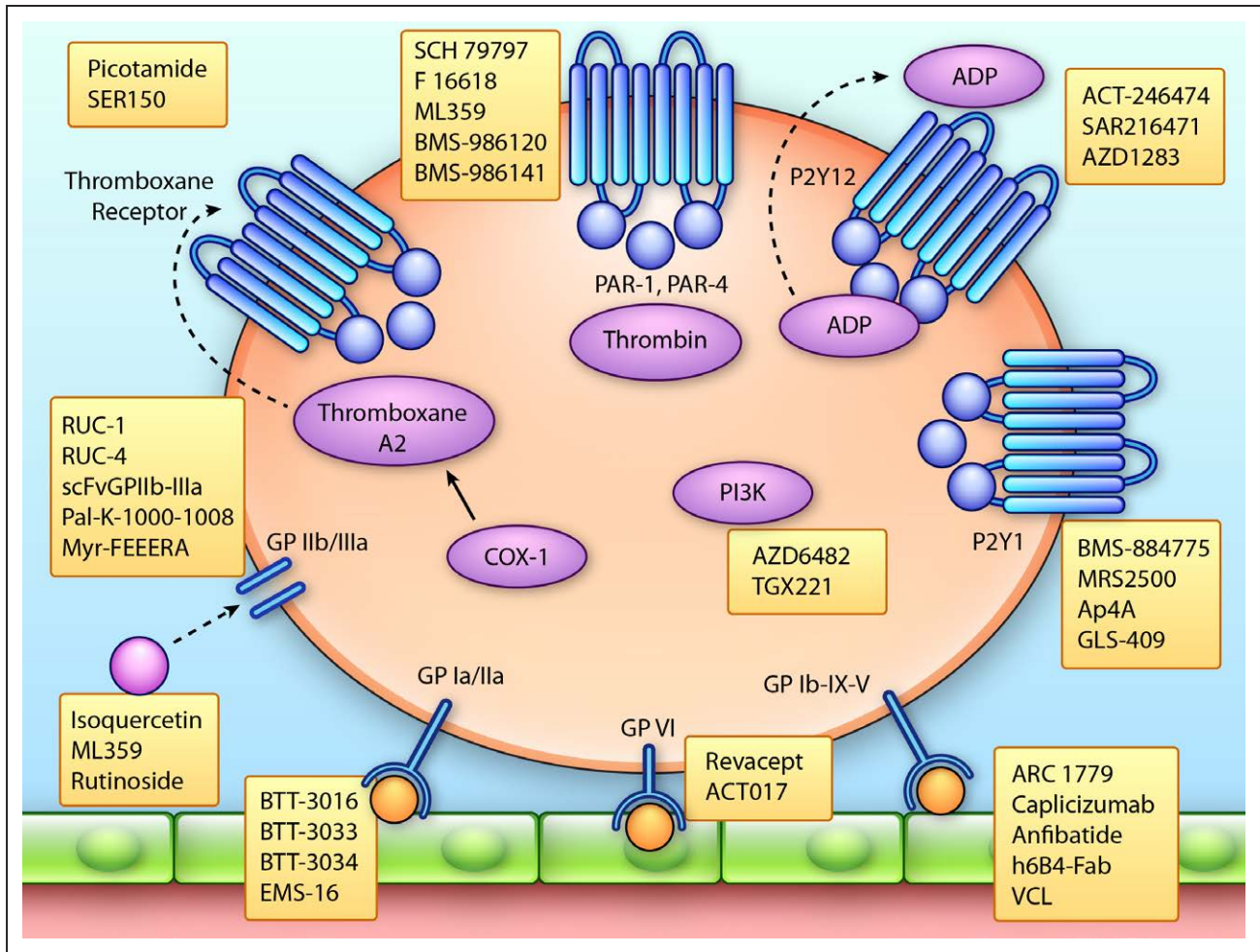
Phosphatidylinositol 3 Kinase B

PI3KB (phosphatidylinositol 3 kinase B) is a lipid kinase with important functions in signaling pathways downstream of platelet receptor activation and for mediating platelet activation at sites of thrombus propagation. AZD6482 is a novel PI3KB inhibitor that has undergone preclinical and early clinical (phase I) study to determine its general safety and tolerability.[49,50] In animal studies using a canine model, AZD6482 produced antithrombotic effects without any increase in bleeding time or blood loss. These effects extended to healthy human volunteers, and the drug demonstrated moderate platelet inhibition at levels between aspirin and clopidogrel.[49,50] The beta isoform-selective PI3K inhibitor, TGX221, was shown to decrease cyclic flow reductions in a preclinical carotid artery stenosis model, without bleeding sequelae.[51] Recent evidence suggests that P13KB also has a critical role in maintaining the integrity of a formed thrombus in an environment of elevated shear stress, and thus, inhibition of this pathway may theoretically increase the risk of thrombus embolization, although the clinical impact of this finding is yet uncertain and requires additional study.[52]

Protein Disulfide-Isomerase

PDI (protein disulfide-isomerase) exist in platelet granules and the platelet surface where they are involved in thrombosis through their action on several intravascular targets.[53,54] PDI inhibitors in preclinical studies have been shown to reduce platelet aggregation and reduce thrombus formation under flow conditions, and have been shown to protect mice from carotid artery occlusion.[54] The PDI inhibitor isoquercetin underwent phase I clinical study and was shown to diminish platelet-dependent thrombin generation by blocking generation of platelet factor Va.[55] Isoquercetin is currently in phase II/III clinical study to evaluate its potential role in preventing venous thrombosis in patients with pancreatic, nonsmall cell lung, or colorectal cancer (http://www.clinicaltrials.gov. Unique identifier: NCT02195232). The primary end point of the phase III portion of the study is the cumulative incidence of venous thromboembolic disease. A clinical role for isoquercetin and other PDI inhibitors in preventing arterial thrombosis in humans has not yet been established. Additional PDI inhibitors which have been shown to inhibit platelet aggregation and are not cytotoxic include rutinoside and the more potent, small molecule inhibitor ML359.[56]

Novel GP IIb/IIIa Receptor Antagonism

GP IIb/IIIa is a highly abundant platelet receptor and is the binding site for fibrinogen. GP IIb/IIIa has an essential role in platelet adhesion and aggregation. Current therapies targeting GP IIb/IIIa receptors are highly potent with a bleeding risk that limits their clinical utility for long-term therapy. However, it is now recognized that GP IIb/IIIa exists in both low- and high-affinity states. On platelet activation, signaling from within the platelet induces conformational changes of the receptor (inside-out signaling), allowing it to bind its primary ligand, soluble fibrinogen. Once a ligand binds to the GP IIb/IIIa receptor, so-called outside-in signaling is thought to mediate intracellular events which allow thrombus propagation and are

Figure 2. Novel and emerging antiplatelet therapies and their targets. Several additional platelet receptors and pathways have been identified as possible drug targets with the goal of maintaining anti-platelet efficacy while minimizing bleeding risk. Among these are inhibitors of PI3KB (phosphatidylinositol 3 kinase B), a lipid kinase which functions in downstream signaling pathways to mediate platelet activation and thrombus propagation. Additionally, targets include PDI (protein disulfide-isomerase), novel GP (glycoprotein) IIb/IIIa inhibitors, PAR (protease activating receptor)-4 antagonists, P2Y1 inhibitors, and inhibitors of GP Ib and GP VI mediated pathways. Ap4A indicates diadenosine tetraphosphate; and COX, cyclooxygenase.

distinct from those which allow hemostasis. Current GP IIb/IIIa inhibitors, which are ligand mimetic agents, may paradoxically potentiate platelet activation through this mechanism.

scFv (single-chain variable fragments) have been developed which target the GP IIb/IIIa receptor specifically in its high-affinity configuration.[57,58] This strategy can block fibrinogen from binding to the receptor without potentiating outside-in signaling. In murine models and ex vivo primate models, these agents demonstrated potency similar to ligand mimetic agents without increases in bleeding. In addition, intracellular inhibitors of GP IIb/IIIa have been developed which disrupt integrin activation and switching to the high-affinity state or which can specifically inhibit outside-in signaling.[59]

Separately, the drugs RUC-1 and RUC-4, identified through high throughput screens for small molecule inhibitors of fibrinogen binding, are specific for the alpha IIb subunit of the GP IIb/IIIa receptor. These agents maintain the receptor in a low-affinity state, unable to bind its major ligand fibrinogen. Studies of RUC-4 have demonstrated that it does not induce conformational changes or platelet activation through outside-in signaling. Unlike a strategy of using scFvs, RUC-4

affects all platelets because its binding is not dependent on the receptor being in an active state, and evidence on its safety profile, particularly with respect to bleeding risk, is lacking at this time.[60]

These agents represent a renewed interest in the potential of GP IIb/IIIa antagonists to provide therapeutic antiplatelet benefits with an improved safety profile.[61]

PAR-4 Antagonists

Despite the efficacy of PAR-1 antagonists, their clinical utilization has been limited because of concerns for significant bleeding. Additional PAR-1 specific antagonists, including SCH 79797 and F 16618, remain under development.[62,63] However, thrombin activates platelets through its action on both PAR-1 and PAR-4. There has been growing interest in the role of PAR-4 receptor as a target for platelet antagonism. PAR-4 expression is highly dynamic and is altered by numerous thrombotic and inflammatory stimuli. The PAR-4 specific antagonist BMS-986120 completed a phase I clinical trial where it was demonstrated to provide selective and reversible PAR-4 antagonism and platelet aggregation. Ex

vivo total thrombus area was significantly reduced, driven by reductions in platelet-rich thrombus deposits.[64] Additionally, the drug was shown to have no effect on thrombus formation at low shear conditions and did not demonstrate an increase in coagulation time. An additional PAR-4 specific antagonist, BMS-986141, was also developed with greater potency than BMS-986120. BMS-986141 was studied for reduction of stroke recurrence in a phase II clinical study among patients with history of stroke or TIA already on aspirin (http://www.clinicaltrials.gov. Unique identifier: NCT02671461). The results are pending at this time.

An additional class of PAR-1 inhibitors, called paramodulins, have been developed which target the cytoplasmic face of PAR-1 without modifying the ligand binding site. This distinction may allow paramodulins to maintain the cytoprotective effects of PAR-1 signaling.[65,66] This is a significant distinction from orthosteric antagonists, such as vorapaxar or atopaxar, which inhibit all signaling downstream of the PAR-1 receptor.[67–69]

GP VI Mediated Adhesion Pathways

GP VI mediates platelet activation through binding of subendothelial collagen. Collagen binding promotes crosslinking of GP VI receptors, which facilitates platelet aggregation and activation through release of platelet agonists, such as ADP and TxA2, and activation of the GP IIB/IIIa receptor. Additionally, both experimental blockade and genetic deficiency of GP VI lead to reduced thrombus formation without major bleeding complications.[70] For these reasons, GP VI is a site of significant interest as an antiplatelet target.

Revacept is a fusion protein comprising the extracellular domain of GP VI and a human Fc immunoglobulin region. It can bind immobilized collagen and prevent platelet adhesion and activation. A phase I clinical study of Revacept demonstrated dose-dependent inhibition of collagen-induced platelet activation without an increase in bleeding time.[71] Studies in animal models suggest that Revacept may reduce thrombus formation and improve vascular endothelial function.[72] In mouse cerebral infarct models, the addition of Revacept to varying doses of recombinant tissue plasminogen activator improved efficacy compared with r-tPA (recombinant tissue-type plasminogen activator) alone, without an increase in intracranial bleeding.[73] It has also been suggested that Revacept may have a role as part of DAPT regimens, in addition to aspirin or P2Y12 receptor antagonist and has been shown to improve platelet inhibition as an adjunct to aspirin or ticagrelor.[74] Phase II clinical studies of Revacept in patients with stable CAD (http://www.clinicaltrials.gov. Unique identifier: NCT03312855) and symptomatic carotid stenosis (http://www.clinicaltrials.gov. Unique identifier: NCT01645306) are currently underway.

ACT017 is an additional GP VI inhibitor currently under investigation. ACT017 is a humanized Fab fragment with high specificity and affinity for GP VI. It has undergone a dose escalation study and was shown to be effective in inhibiting collagen-induced platelet aggregation ex vivo after injection into a macaque.[75] Additionally, there was no evidence of thrombocytopenia or excess bleeding with its use.

The GP Ia/IIa receptor for collagen also remains another target of interest for antiplatelet therapies. Inhibitors of GP Ia/IIa may reduce thrombus formation after arterial injury in preclinical models.[76]

GP Ib/IX/V inhibition

The GP Ib/IX/V receptor binds to von Willebrand factor (vWF) during injury and under conditions of high shear stress, allowing early platelet adhesion to the subendothelium. The GP Ib/IX/V receptor and vWF have been proposed as additional antiplatelet targets, although antibodies developed against vWF have demonstrated unacceptably high rates of bleeding, leading to their discontinuation. ARC1779 is an intravenous oligonucleic acid aptamer that binds vWF and had entered phase II/III clinical study in patients undergoing carotid endarterectomy, but the study was halted because of lack of funding. Available data suggested that the drug reduced carotid embolic events but was associated with an excess of bleeding.[77] The single-domain antibody caplacizumab, which also binds vWF, was studied in a phase I/II clinical trial among patient with stable angina and shown to improve peripheral endothelial function.[78] The drug was also studied as an adjunct to DAPT in patients with ACS but was found to have prohibitive bleeding risk. However, caplacizumab has emerged as a potential treatment for patients with thrombotic thrombocytopenic purpura, where it has demonstrated significant reduction in thrombotic complications and is now poised for phase III study.

The snake venom derivative anfibatide is a direct anti-GP Ib antagonist that also inhibits vWF. Anfibatide has been shown to inhibit platelet adhesion and aggregation in preclinical models and was protective of ischemic stroke and reperfusion injury in mice.[79,80] A phase II clinical trial to assess the safety and efficacy of anfibatide in patients with ST-segment–elevation myocardial infarction before PCI is also underway (http://www.clinicaltrials.gov. Unique identifier: NCT02495012).

Additional preclinical agents, directly targeting GP Ib or the vWF binding domain, are under development at this time.

Novel P2Y12 and P2Y1 Inhibition

Novel, direct, P2Y12 inhibitors remain under development at this time, including the highly potent inhibitors ACT-246475, AZD1283, and SAR216471.[81,82] ACT-246475 was associated with less bleeding, higher selectivity, and equivalent antithrombotic efficacy to ticagrelor in rat models and is now undergoing phase II study (http://www.clinicaltrials.gov. Unique identifier: NCT03384966).[83]

The P2Y1 receptor initiates ADP induced platelet aggregation and shape change, whereas P2Y12 activation is responsible for amplification and stabilization. The potential of P2Y1 inhibition as an antiplatelet strategy to reduce bleeding risk has been recently explored. The compound BMS-884775 demonstrated similar efficacy with less bleeding compared with prasugrel in a rabbit model.[84] In a preclinical study, the P2Y1 receptor antagonist MRS2500 was shown to prevent carotid artery thrombosis in cynomolgus monkeys.[85] The potential of combined P2Y1 and P2Y12 receptor inhibition is

also of interest and led to the development of the compound diadenosine tetraphosphate and additional derivative compounds.[86] Of these, GLS-409 has shown significant potential in preclinical studies, where it has been shown to be highly potent, and to improve coronary blood flow recovery in a canine model of unstable angina, with minimal increase in bleeding time.[87,88]

12-Lipoxygenase Inhibitors

12-LOX (platelet 12-lipoxygenase) is an oxygenase predominantly expressed in human platelets. 12-LOX utilizes arachidonic acid as a substrate to form bioactive metabolites that have been shown to play a role in platelet activation and granule secretion.[89] For this reason, there is an interest in the utility of specific 12-LOX inhibitors as antiplatelet therapy. A recent study of the first inhibitor of 12-LOX, ML355, demonstrated dose-dependent inhibition of human platelet aggregation.[89] Ex vivo flow chamber assays confirmed attenuation of platelet adhesion and thrombus formation at arterial shear over collagen in whole blood, with effects comparable to aspirin. Additionally, in a mouse model, oral ML355 treatment impaired thrombus growth in an arteriole thrombus model, with minimal bleeding.

Conclusions

Antiplatelet therapies remain an essential tool to reduce the risk of developing clinically apparent atherothrombotic disease and are a mainstay in the therapy of patients who have established cardiovascular, cerebrovascular, or peripheral artery disease. Strategies to intensify antiplatelet regimens should be complemented by approaches that focus on targeting thrombosis while preserving hemostasis. Several novel antiplatelet therapies which are being developed target a wide range of receptors and signaling pathways that have been unexplored and hold tremendous potential to improve patient outcomes by maintaining antiplatelet efficacy and preserving hemostasis Figure 2. Additional study in human subjects and with randomized trials will clearly be required before such agents can be widely disseminated, but their therapeutic promise gives reason for optimism and excitement in the treatment of atherothrombotic diseases.

Disclosures

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Highlights

- Antiplatelet therapy remains an essential tool to reduce the risk of developing ischemic complications and is a cornerstone of therapy in those with established disease.
- Strategies to reduce atherothrombotic events include intensifying antiplatelet regimens and may be complemented by approaches that focus on targeting thrombosis while preserving hemostasis.
- Several novel antiplatelet therapies are being developed that target a wide range of receptors and signaling pathways which are unexplored clinically and may improve patient outcomes.