Review

Platelet reactivity in dyslipidemia: atherothrombotic signaling and therapeutic implications

Moua Yang[1], Andaleb Kholmukhamedov[2,*]

[1] Division of Hemostasis and Thrombosis, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02115, USA

[2] Versiti Blood Research Institute, Milwaukee, WI 53226, USA

*Correspondence: AKholmukhame@ilww.com; AKholmukhamedov@Versiti.org (Andaleb Kholmukhamedov)

DOI:10.31083/j.rcm.2021.01.256

Submitted: 23 November 2020 Revised: 05 January 2021 Accepted: 06 January 2021 Published: 30 March 2021

The risks for adverse thrombotic events, including myocardial infarction, stroke, and deep vein thrombosis, are markedly increased in dyslipidemia and other metabolic disorders and are the major cause of death worldwide. Recent evidence points out that increased thrombotic risk in dyslipidemia is mediated by platelets circulating in a pre-activated state. The mechanisms of platelet reactivity in this setting are multifaceted including platelet activation by classic agonist receptor signaling as well as platelet sensitization by pattern recognition receptors. Elevated platelet counts in dyslipidemia due to dysregulation in hematopoiesis also contribute to the overall thrombotic phenotype. Despite recent advancements in antiplatelet and anticoagulation therapies, recurrences of adverse thrombotic events remain to be a large clinical burden. In the light of new knowledge, understanding mechanisms that drive pathologic thrombosis in dyslipidemia, the antithrombotic approach shall be revisited. Here, we discuss potential therapeutic avenues based on the overview of platelet signaling mechanisms that contribute to a prothrombotic phenotype in dyslipidemia.

Keywords

Dyslipidemia; Platelet; Thrombosis; Antiplatelet therapy

1. Introduction

Adverse thrombotic events account for 1 in for 4 deaths worldwide and represent a major clinical burden [1]. Inappropriate platelet activation in this setting is one of the primary drivers of adverse thrombotic events and is an attractive therapeutic target in coronary artery disease (CAD). Indeed, antiplatelet agents together with the anticoagulant regimen remain to be the mainstream treatment to prevent arterial thromboses. Clinically used antiplatelet drugs can be divided into five categories by their mechanism of action: antagonists of integrin αIIbβ3, antagonists of PAR1, inhibitors of purinergic $P2Y_{12}$ receptors, inhibitors of cyclooxygenase, and less commonly used inhibitors of phosphodiesterase. Yet the utility of antiplatelets can increase the risk for bleeding complications and are context-dependent in preventing the risk for recurrent thrombotic events [2, 3]. The coagulation pathways are also attractive targets to decrease thrombotic events [4]. Direct oral anticoagulants and vitamin K antagonism show efficacy in preventing potential coagulopathy in these conditions but require careful administration and monitoring to prevent the risk for bleeding complications [5].

The dyslipidemic state is a metabolic disorder that promotes the risk of thrombosis and is characterized by dysregulated levels of cholesterol, triglycerides, and dietary fatty acids [6]. The association between dyslipidemia and platelet activation is well-supported by *in vitro* mechanistic data of platelet reactivity and major adverse cardiac events in patients with coronary artery disease [7–9]. Specifically, *in vitro* studies with platelets isolated from individuals with coronary artery disease and/or familial hypercholesterolemia, as well as atherogenic-prone mice show increase reactivity when activated by classic platelet agonists [7, 9, 10]. These studies are also supported by *in vivo* study of dyslipidemia in atherogenic mice (e.g. the *apoE* or *ldlr* null mice on a "Western" high fat- and high-cholesterol diet) where a prothrombotic phenotype could be observed when thrombosis is induced in the carotid artery by the chemical oxidant ferric chloride [7]. The dyslipidemic state also enhances the risk for venous thrombotic events, including deep vein thrombosis, as suggested by recent clinical data of a cohort of individuals with metabolic syndrome [11]. As such, there is much interest in understanding the pathways that promote platelet activation and coagulation in dyslipidemia to prevent thrombotic events.

An active area of current research is understanding the connection between circulating "factors" that promote low-key platelet activation that is synergized with classic agonist-induced platelet activation signaling that would further augment platelet response. At the mechanistic level, much of the focus has been on specific pattern recognition receptors present on platelets and how these pathways crosstalk with classic agonist stimulation. As these "non-classical" signaling pathways are induced only during the dyslipidemic state, understanding these pathways could pinpoint therapeutic targets for atherothrombosis without compromising hemostasis.

In addition to sensitizing platelet activation, dyslipidemia also seems to result in thrombocytosis, which ultimately elevates the risk for adverse thrombotic events [12]. The mech-

anisms by which there is an increased platelet number in dyslipidemia is poorly understood compared to the mechanisms of platelet activation in this condition. Thrombocytosis in these settings is likely linked to the sensitivity of the bone marrow niche to cholesterol. Yet, both heighten platelet reactivity and thrombocytosis contribute to the overall atherothrombotic risk observed in dyslipidemia.

In this review, we discuss the mechanisms of platelet activation in thrombosis and hemostasis with emphasis on the GPVI and PAR pathway. We then discuss the mechanisms of platelet activation in dyslipidemia with a particular focus on the procoagulant phenotype induced by the pattern recognition receptor CD36. We further discuss the current understanding between dyslipidemia and hematopoiesis with discussions on thrombocytosis and reticulated immature platelets. Finally, we outline potential therapeutic approaches for antiplatelet therapy in dyslipidemia based on current knowledge of atherothrombosis.

2. Mechanisms of platelet activation and their pro-aggregatory function

Platelets are cell "fragments" that circulate in the blood in a quiescent resting state and are best known for their roles in thrombosis and hemostasis. Upon vessel damage exposure of extracellular matrix and tissue factor promotes platelet activation and clot formation through a series of events. These events include platelet adhesion to the site of vessel injury, platelet spreading and aggregation, as well as the transition of a subset of platelets to procoagulant phenotype. In hemostasis, these events are necessary to prevent blood loss, whereas thrombotic complications are associated with "unchecked" mechanisms leading to occlusion of the blood vessel.

Damage to the vessel wall causes exposure of extracellular matrices, including thrombogenic collagen and tissue factor. Tissue factor expression will activate the extrinsic pathway of coagulation to promote thrombin generation, whereas the exposed extracellular matrix promotes platelet adhesion at the site of injury by a repertoire of platelet adhesion molecules. As platelets get arrested at the site of the injury, they become activated. Classic platelet activation pathways are agonist dependent with two major subgroups: glycoproteins and G-protein coupled receptors (GPCR). We focus on two major types of receptors here, the collagen receptor glycoprotein VI (GPVI) and protease-activated receptors (PARs) 1 and 4 as potent platelet activation pathways that are intimately linked to procoagulant functions.

GPVI is a member of the immunoglobulin superfamily and is constitutively associated with Fc Receptor (FcR) γ-chain. GPVI is a receptor for collagen and is expressed in both megakaryocytes and platelets. Recognition of collagen by GPVI promotes Src family kinase-mediated phosphorylation of the immunoreceptor-based activation motif (ITAM) on FcR γ-chain and recruitment of the non-receptor tyrosine kinase spleen tyrosine kinase (SYK) [13, 14]. Syk recognition ultimately leads to activation of phospholipase Cγ2, activation of protein kinase C (PKC), and cytosolic calcium mobilization. The latter is essential for integrin αIIbβ3 activation, platelet granule release, and externalization of procoagulant phosphatidylserine (PSer) [15–17]. Recent evidence suggests that individuals with homozygous insufficiency of GPVI display several defects in platelet activation markers. In a study by Nagy *et al.*, GPVI deficiency (both hetero- and homozygous) displays defects in platelet spreading and PSer exposure [18]. Yet, no differences were observed in platelet adhesion onto collagen-coated surfaces nor other extracellular matrix-coated surfaces in GPVI homozygous or heterozygous deficiency [18]. This suggests that GPVI may participate in selective platelet functions beyond its classic role as a platelet adhesion receptor. Furthermore, GPVI was shown recently to be important in promoting platelet aggregation of pre-formed thrombus independent of thrombin [19]. Using a combination of *in silico* modeling, *ex vivo* platelet functional studies, and *in vivo* intravital microscopy, Ahmed *et al.* showed that inhibitory Fab fragments to GPVI promoted disaggregation of platelets of a growing thrombus. The authors posed that the disaggregation effect is selective to the inhibitory action of the Fab fragment ACT017 on GPVI's recognition of fibrinogen. Although the studies are limited to anticoagulated blood where thrombin generation could not be established, the effect of inhibiting GPVI on disaggregation events are yet to be understood in the context of thrombogenesis.

The effect of GPVI-ITAM signaling is not limited to its contribution to a thrombus that has already formed. GPVI-ITAM signaling was also shown to promote the generation of reactive oxygen species (ROS) through activating NADPH oxidase, a multisubunit complex on the cell membrane that transfers reducing equivalents from NADPH to molecular oxygen [20–22]. Reactive oxygen species by NADPH oxidase and other sources are an important modulator of procoagulant PSer externalization [23, 24]. However, the signaling mechanisms of activating NADPH oxidase by classic platelet agonists or its function in thrombosis and hemostasis are less clear. An elegant study by Sonkar *et al.* showed that targeted deletion of NADPH oxidase in mice or platelets from chronic granulomatous disease (CGD) with no functional NADPH oxidase still responds to platelet agonists, suggesting that it may not be required [25]. Yet, other reports indicate that NADPH oxidase is a functional source of oxidant generation in platelets that are agonist-specific [21, 22, 26–28]. The role of NADPH oxidase for platelet activation, as well as its role in generating ROS by classic agonists, requires further investigation.

Thrombin generation at the site of vessel injury promotes potent platelet activation [29]. Thrombin generation is the result of proteolytic activation of its zymogen prothrombin by the prothrombinase complex assembled downstream of intrinsic and extrinsic coagulation pathways. Thrombin promotes activation of the protease-activated GPCRs by cleaving the tethered N-terminal tail of the protein. There are 4 members of the PAR families with human platelets express-

ing PAR 1 and 4. PAR1 signaling is transient whereas PAR4 signaling is sustained and necessary to achieve thrombus stability [30, 31]. PARs are coupled to either G_q or $G_{12/13}$. Coupling to $G_{12/13}$ protein will trigger platelet shape change as a part of platelet aggregation through guanine nucleotide exchange factor Rho. When coupled to G_q protein, PARs will induce calcium mobilization through phospholipase Cβ-dependent generation of inositol triphosphate and diacylglycerol and downstream platelet activation.

3. Mechanisms of procoagulant platelet formation

Platelets support coagulation during thrombus formation by providing a negatively charged procoagulant surface for tenase and prothrombinase complexes [32, 33]. Thrombin not only promotes platelet activation as described above but also cleaves soluble fibrinogen to insoluble fibrin and thus stabilizes the primary platelet plug [29]. Procoagulant platelets are the subpopulation of platelets that link primary and secondary hemostasis and are also described in the literature by a plethora of nomenclature, including COAT and coated platelets, ballooned platelets, necrotic platelets, and many others based on their different morphologic and functional characteristics. Nonetheless, one of the undisputed characteristics of this subpopulation of activated platelets is the externalization of anionic phosphatidylserine (PSer) [34, 35]. We refer the readers to additional excellent reviews on the characteristic properties of procoagulant platelets in [35–37].

Potent platelet activation promotes the loss of membrane symmetry through multiple intracellular cues that ultimately trigger the activation of specific scramblases for PSer exposure. PSer is physiologically maintained in the inner leaflet of the cellular membrane until 'flipped' by scramblases to the exterior milieu [38]. Several anoctamins mediate PSer externalization with anoctamin 6, or TMEM16F, as the most notable to platelet PSer externalization [39]. The specific mechanisms of TMEM16F activation in activated platelets are still unclear but were shown to follow a calcium-dependent mechanism [40]. Studies investigating the regulation of PSer exposure by scramblases point to the fact that it is directly linked to mitochondrial function [39, 41–43].

Another widely accepted feature of procoagulant platelets is the fact that they are maximally generated in the settings of GPVI and PAR1/4 co-stimulation. This is classically utilized by multiple laboratories to study platelet procoagulant functions [16, 23, 44–47]. At the receptor level, abrogating GPVI interaction with collagen or the snake venom convulxin (CVX, a higher affinity GPVI ligand than collagen) or the absence of the associating FcRγ chain prevents procoagulant platelet formation as assessed by PSer externalization [48]. The downstream signaling events for GPVI to promote procoagulant PSer are presumably through the signaling by Src family kinases, which in turn activates PLCγ2 leading to intracellular calcium mobilization [49]. Src involvement has largely been attributed to the use of tyrosine kinase inhibitor dasatinib, which reduces the percentage of platelets positive for PSer externalization and clot retraction when induced by the GPVI agonists collagen or convulxin [49]. The role of calcium-induced PSer externalization is not restricted to the GPVI pathway. Robust activation by PARs also enhance calcium mobilization required for PSer externalization through a G_q-coupled phospholipase Cβ-dependent mechanism [35]. Dual agonist stimulation by both thrombin and collagen increases cytosolic calcium to the concentrations observed in micromolar ranges, which is well enough to perturb calcium homeostasis and mitochondrial function [50]. Calcium mobilization in this setting is mediated by both intracellular calcium mobilization and extracellular calcium import. Uptake of extracellular calcium is a coordinated interplay between the ORAI1 and STIM1 proteins [51]. Deficiency of ORAI1 and/or STIM1 leads to diminished procoagulant potential by multiple platelet agonists [51].

Platelet mitochondria are exquisitely sensitive to calcium levels. The mitochondrial calcium uniporter (MCU), localized on the inner mitochondrial membrane, was shown to be a conduit for mitochondrial-mediated PSer externalization [16]. This event is triggered by the increase in mitochondrial calcium intake from the cytosol as a regulatory mechanism to normalize cytosolic calcium. MCU is coordinated with multiple mitochondrial membrane proteins [52]. Cryo-EM structure of MCU has recently been published suggesting a tightly regulated calcium coordinated process for calcium uptake in the mitochondria [52–54]. It is known that the MCU activity favors the generation of the mitochondria permeability transition pore (mPTP) in platelets in part by increasing the activity of peptidylprolyl isomerase cyclophilin D (CypD) [16]. Genetic ablation of CypD in platelets and inhibition of CypD with cyclosporin A abrogates PSer externalization to baseline levels, indicating CypD is a key component regulating platelet procoagulant activity [23, 41]. MCU activity was also shown to be regulated not only by its substrate levels but also post-translationally by phosphorylation on the N-termini [55]; however, this has yet to be studied in platelets.

Although the mechanisms of canonical platelet activation and procoagulant activity in hemostasis and thrombosis are an active area of research, new data on non-canonical mechanisms of platelet procoagulant functions, such as observed in metabolic disorders like dyslipidemia, support a complex regulation of PSer externalization.

4. Heightened platelet reactivity in dyslipidemia

In dyslipidemia, platelets are sensitized to activation by oxidized phospholipids found in low-density lipoprotein (LDL) particles. Phospholipids are particularly sensitive to oxidation [56]. Oxidized phospholipids in this setting are generated during the inflammatory processes of plaque formation and circulate in micromolar ranges [7]. Oxidized

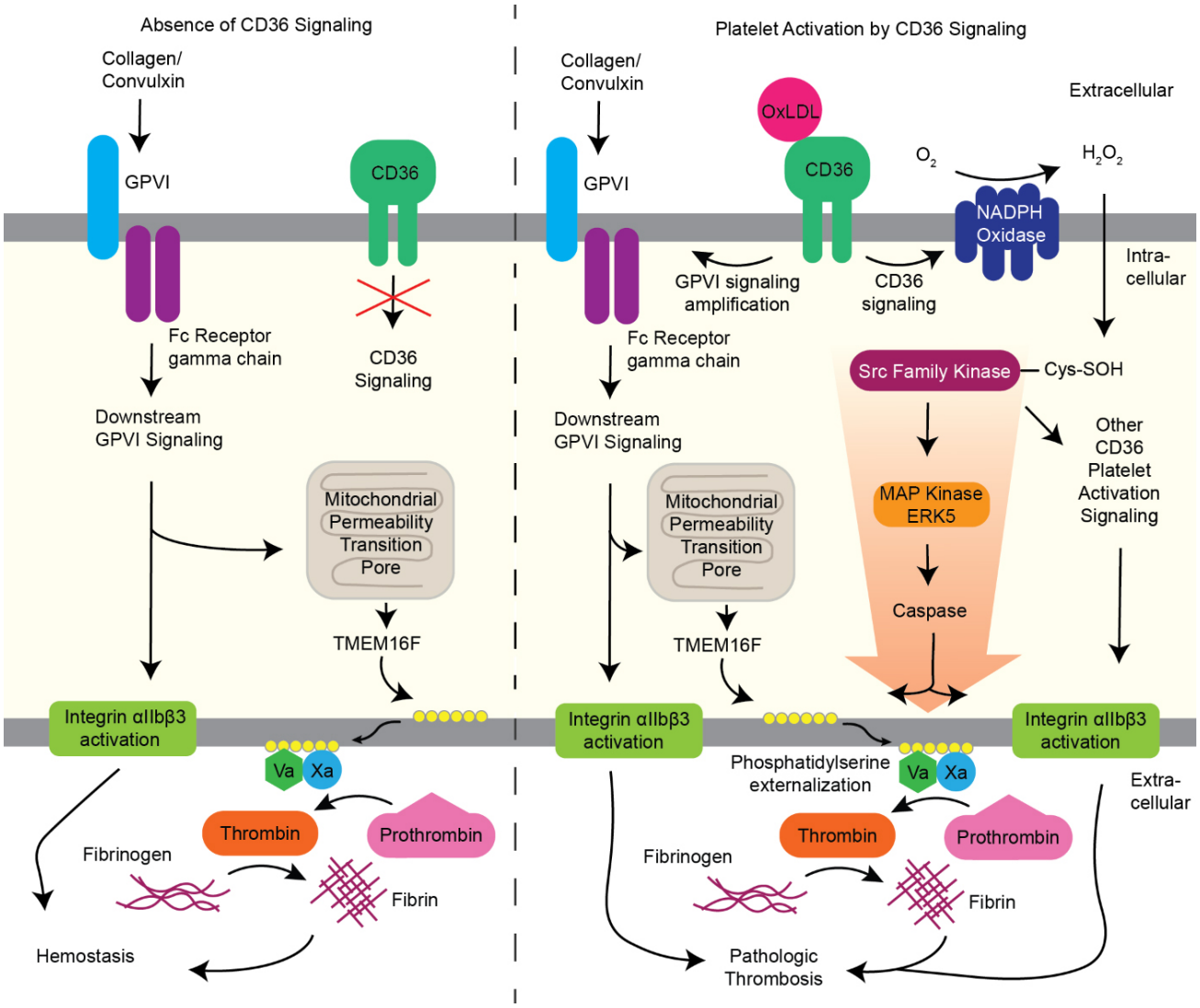

Fig. 1. Platelet CD36 prothrombotic signaling in dyslipidemia. Platelet CD36 promotes thrombosis in dyslipidemia by two different mechanisms: 1) direct platelet activation by multiple signaling pathways including a pathway involving the amplification of molecular signaling downstream of the collagen receptor GPVI; 2) indirectly promoting platelet activation by dampening the major platelet inhibitory signaling pathways. In the direct platelet activation pathway, CD36 sensitizes platelet activation to collagen by promoting reactive oxygen species generation by NADPH oxidase. It is the hydrogen peroxide reactive oxygen species that promote cysteine sulfenylation of multiple platelet proteins, including Src family kinases. Cysteine sulfenylation of Src family kinases maintains kinase activity to activate downstream signaling pathways, including the redox-sensitive MAP kinase ERK5. ERK5 then link CD36 signaling to two functional phenotypes: pathophysiologic thrombosis by promoting integrin αIIbβ3 activation and caspase-dependent phosphatidylserine externalization for platelet procoagulant activity. In the absence of CD36 signaling, the GPVI signaling pathway promotes physiologic thrombosis. CD36 signaling was shown to dampen the cGMP and cAMP inhibitory signaling and therefore promote platelet activation by physiologic agonists.

phospholipids are a major risk factor for thrombotic events as they sensitize platelets to activation by a specific pattern recognition receptor known as CD36.

The best-characterized receptor for oxidized lipids in dyslipidemia is the scavenger receptor CD36, a multi-functional 88 kDa protein that is also known as platelet glycoprotein IV or fatty acid translocase [57, 58]. The crystal structure for CD36 has not been yet solved; however, homology modeling based on its related receptor LIMPII were hypothesized [59].

CD36 has two short intracellular tails, two transmembrane domains, and a very large and heavily N-glycosylated extracellular domain [60]. CD36 "senses" several ligands as part of its innate immune function. These include oxidized lipids in oxidized LDL particles [7], the proinflammatory S100A8/A9 family of calcium-binding proteins [61], proteins containing the thrombospondin type I-repeat domains [62], advanced glycated end products [63], microparticles from damaged cells [64], free fatty acids [65], high-density lipoprotein particles [66], and staphylococcal lipoteichoic acids [67]. The signaling pathways induced by CD36 are context- and cell-dependent [68]. In platelets CD36 is responsible for two distinct pathways, as demonstrated in Fig.

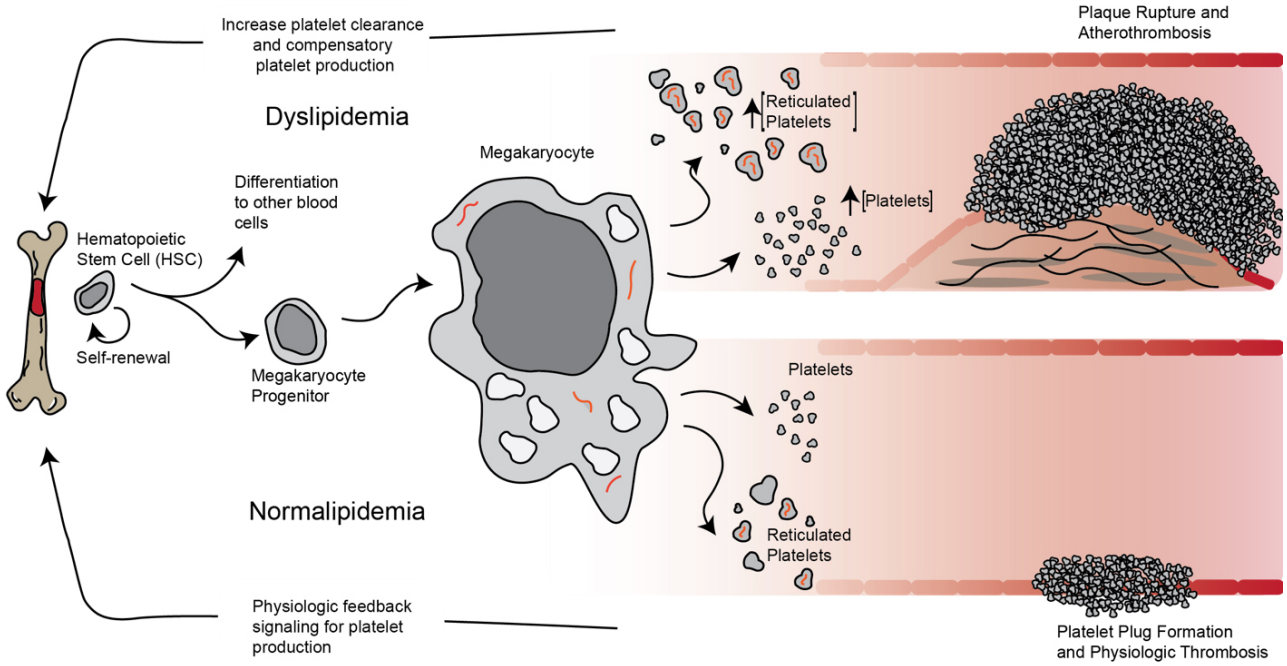

Fig. 2. Reticulated platelets and increase platelet production are risk factors for atherothrombosis. The bone marrow niche is sensitive to cholesterol in dyslipidemia. Reticulated platelets are larger immature platelets produced from megakaryocytes. In metabolic disorders, increase reticulated platelet production and platelet count contribute to an overt prothrombotic phenotype that increases the risk for clinically significant thrombotic events. The mechanisms for increase reticulated platelet production and platelet count are not well-understood but may be linked to a compensatory mechanism for platelet clearance. In normolipidemia, platelets contribute to physiologic thrombosis as part of the mechanism to form a platelet plug. Normal platelet production is governed by the platelet half-life in circulation.

1: 1) platelet activation by circulating oxidized lipids that further synergize with activation pathways of 'classic' agonists, and 2) disabling both the caMP-PKA [69, 70] and cGMP-PKG inhibitory pathways [71].

CD36 is highly expressed (up to 20,000 copies per platelet) on the platelet surface [72, 73]. Biochemical studies by Huang *et al.* demonstrate that upon activation, platelet CD36 associates with the Src family members Fyn, Lyn, and Yes [74]. These studies were further verified by independent labs looking at Src family members activated by CD36 in different cell types including endothelial cells [75], monocyte/macrophages [76], and platelets [77]. In the platelet system, the best characterized Src family members activated by CD36 are Fyn and Lyn, with Fyn being the predominant one. Src family kinases connect CD36 to platelet activation through Vav guanine nucleotide exchange factors [78], activation of MAP kinases JNK1 [77] and ERK5 [79], the Rho/ROCK signaling module for cytoskeletal rearrangement [80], and a pathway leading to the activation of Protein Kinase C, Phospholipase Cγ2, and generation of superoxide radical anion by NADPH oxidase [70, 71, 79]. Recent studies show that superoxide radicals generated by CD36 signaling disproportionate to the two-electron oxidant hydrogen peroxide [81]. It is the hydrogen peroxide that oxidizes cysteines on multiple proteins within the cell, including Src family kinases to maintain kinase activity [81, 82]. In this context, the transient cysteine sulfenic oxoform was detected using benzothiazine-based carbon nucleophiles [83, 84]. The sulfenic acids generated is important for platelet-mediated proaggregatory and procoagulant functions that support arterial thrombosis in dyslipidemia [81]. The role of oxidant generation by CD36 also participates in regulating the platelet inhibitory pathways. In particular, oxidant generation by NADPH oxidase in this setting blunt the inhibitory platelet cGMP pathway [71]. In combination with CD36's ability to promote platelet hyposensitivity to prostacyclins [69, 70], these multifaceted approaches lower the threshold for platelet activation.

MAP kinases are a family of serine/threonine kinases that are activated in conditions of cellular stress, proliferation, growth, apoptosis, and differentiation and are highly expressed in platelets [85]. Platelet MAP kinase ERK5 was shown to be activated in conditions of greatly elevated ROS generation, such as during the ischemic conditions of myocardial infarction [86, 87]. Pharmacologic inhibition of ERK5 in platelets or platelet-specific ERK5 deficiency show protection from the elevated platelet activation profiles observed in ischemia compared to platelets treated with vehicle control or in ERK5-expressing animals, respectively [86]. The redox regulation of ERK5 activation is not limited to ischemic conditions; platelet ERK5 is also activated in conditions of increased ROS generation in dyslipidemia [24, 79].

Platelet CD36 signaling generates ROS through NADPH oxidase and in this setting ERK5 is activated as a redox signaling node for platelet pro-aggregatory functions.

MAP kinase ERK5 activation by CD36 signaling is not limited to proaggregatory functions. CD36-mediated ERK5 activation induces procoagulant PSer [24] that is distinct from the physiologic pathways of PSer externalization driven by CypD and mitochondrial permeability transition [16, 23, 88] or the PSer externalization pathway driven by apoptotic caspases for platelet clearance [89, 90]. CD36 and ERK5 sensitize the GPVI signaling pathway for PSer augmentation. The procoagulant nature of this sensitization by CD36 and ERK5 requires Src family kinases, hydrogen peroxide, and apoptotic caspases as pharmacologic inhibition of Src, scavenging hydrogen peroxide and preventing caspase activation prevented the PSer profiles observed with oxidized lipids [24, 79]. In line with the role of hydrogen peroxide and ERK5 being important for CD36-mediated PSer externalization, cysteine sulfenylation of Src family kinases upstream of ERK5 activation supports PSer externalization [81]. CD36 and ERK5-mediated PSer externalization enhance factor tenase and prothrombinase activation and subsequent fibrin deposition both *ex vivo* and *in vivo* [24]. These mechanisms may describe the thrombin generation phenotype mediated by platelets in dyslipidemic patients [91].

Platelet CD36-mediated procoagulant functions can vary depending on the settings. In a separate study, Dohrmann *et al.* showed that CD36-fibrin interaction propagates FXI-dependent thrombin generation of human platelets in advanced nephropathies [92]. The authors report that in chronic kidney disease, thrombin induces thrombin generation that was prevented in the presence of a CD36 blocking antibody. FXI, fibrin, GPIbα and Src family kinases are also required for this CD36-dependent thrombin generation. In addition to other pathologic conditions, specific CD36 ligands could link the receptor to hemostatic capabilities. Recently, it was shown that platelet-derived thrombospondin-1 mediates hemostasis *in vivo* through a CD36-associated mechanism [93]. Thrombospondin-1 is a CD36 ligand and was shown to augment platelet activation [94, 95]. In this study, targeted genetic deletion of thrombospondin 1 in platelets prolonged bleeding time in mice that could be rescued by the infusion of wild-type platelets [93]. Mechanistically, the authors proposed that thrombospondin-1 dampens the cAMP inhibitory signaling pathway. Collectively these studies suggest that the mechanism of CD36-mediated platelet procoagulant functions may be context-dependent.

Additional platelet scavenger receptors could regulate platelet reactivity in dyslipidemia. The closely-related receptor family member scavenger receptor A-1 (SRA-1) was shown to promote platelet activation in mouse and human platelets through a p38 MAP kinase-dependent mechanism [96]. In this study, platelet activation by SRA-1 by oxidized lipids could involve CD36 as blocking CD36 or the use of CD36-deficient mouse platelets inhibits platelet spreading on immobilized collagen [96]. The selective recognition of oxidized lipids by SRA-1 compared to CD36 is directly related to the extent of lipoprotein oxidation as lipoproteins exist in different oxidized states [56].

Platelet reactivity in dyslipidemia is regulated by specific oxidized phospholipids present in the circulation that are coordinated between CD36 with other innate immune receptors. The signature work by Podrez and colleagues indicated that it is predominantly the oxidized phosphatidylcholine (PC) phospholipid species termed oxPC_{CD36} that are high-affinity ligands for CD36 and are present in micromolar levels in the plasma of patients with CAD or from atherogenic-prone mice fed a high fat and high cholesterol diet [7]. However, subsequent work also indicated that a novel oxidized derivative of the phosphatidylethanolamine (PE) phospholipid is also present in dyslipidemia [97]. These oxidized PE derivatives, called carboxyalkylpyrrole-PEs, could be recognized by toll-like receptors to promote platelet activation [97]. It is likely that in dyslipidemia toll-like receptors and CD36 coordinate to promote platelet activation for a prothrombotic state as was proposed by studies showing that CD36 forms a complex with TLR2/6 for oxPC_{CD36}-mediated platelet signaling [98]. These studies suggest a higher-order innate-immune receptor regulation in the context of atherothrombosis. Further work to elucidate the mechanisms by which CD36 promotes platelet proaggregatory and procoagulant functions will be required and will pinpoint regulatory signaling nodes as potential therapeutic targets.

5. Thrombocytosis and "hyperactive" platelet reticulocytes

In dyslipidemia, dysregulated hematopoiesis induced by cholesterol stress also increases platelet counts (thrombocytosis) and reticulated platelet production. The mechanisms of thrombocytosis in this condition are not very well-understood but seem to be an alternative mechanism that increases the risk for atherothrombosis. A schematic of this process is shown in Fig. 2. Platelet production from megakaryocytes is driven by the coordination between thrombopoietin (TPO) and its receptor c-Mpl, a type I cytokine receptor family [99, 100]. Like other type I cytokine receptor family members, activation of c-Mpl promotes Janus kinase 2 (JAK2) and STAT3/5 signaling in hematopoietic stem cells and megakaryocytes [101–103]. Of relevance to platelet production, JAK/STAT signaling by TPO and c-Mpl promotes megakaryocyte maturation, differentiation, proplatelet formation, and the release of platelets [104, 105].

Platelet half-life in circulation is also linked to desialylation and clearance in the liver by the Ashwell-Morell receptors [106] and by PSer externalization by apoptotic caspases [89]. In dyslipidemia, decreased survival of platelets has been linked to the plasma lipid profiles [107]. Although this could be related to desiaylation, it is possible that PSer externaliza-

tion on platelets during dyslipidemia promotes platelet clearance that enhances TPO production and, as a result, reactive thrombocytosis. However, this hypothesis requires experimental evidence. An increase in megakaryocyte ploidy and mean platelet volume, two markers associated with increase platelet production, has been well-documented in hypercholesterolemia [108, 109]. Megakaryocytes were also documented to be delocalized and closer to the bone marrow sinusoids in murine models that could contribute to the overt thrombocytosis phenotype [110]. Furthermore, thrombocytosis could be regulated by cholesterol efflux, as the ATP-binding cassette transporter ABCG4 that is highly expressed in the bone marrows regulate megakaryocyte cholesterol levels, platelet production, and arterial thrombosis [111].

In metabolic disorders, reticulated platelet production is elevated and has been associated with adverse cardiovascular events [112]. Reticulated platelets are larger and are identified by their retention of RNA contents from megakaryocytes [113]. Reticulated platelets contribute to the prothrombotic phenotype observed in dyslipidemia as reticulated platelets are thrombogenic and promote platelet activation supporting atherothrombosis [112]. The mechanisms of reticulated platelet production in dyslipidemia are not clear but may be independent of *de novo* cholesterol synthesis as statin usage in patients with coronary artery disease do not affect reticulated platelet counts [114]. Understanding the mechanisms of cholesterol sensitization in the bone marrow niche and thrombocytosis would identify additional targets in reducing the risk for thrombotic complications in dyslipidemia.

6. Current antiplatelet agents and potential therapeutic avenues

Antithrombotic medications, used to treat arterial and/or venous thrombotic complications, can be classified into two major groups: antiplatelets and anticoagulants. Clinically used antiplatelet agents act on platelet receptors and their intracellular signaling pathways that prevent their activation and subsequent aggregation [115]. As outlined in Fig. 3 and Table 1, there are 5 classes of clinically used antiplatelet agents: 1) inhibitors of cyclooxygenases; 2) purinergic receptor $P2Y_{12}$ inhibitors; 3) PAR1 antagonists; 4) integrin $\alpha IIb\beta 3$ antagonists; and 5) inhibitors of phosphodiesterase (PDE). Most of these medications ultimately inhibit platelet aggregation function, some as direct inhibitors, and some inhibiting molecular aspects of platelet activation leading to integrin activation and subsequent aggregation.

The most used antiplatelet drug is aspirin, an irreversible inhibitor of cyclooxygenase, which inhibits both cyclooxygenase 1 expressed in platelets and cyclooxygenase 2 expressed in endothelial cells [116]. Cyclooxygenases catalyze prostaglandin H_2 generation from the precursor arachidonic acid for the synthesis of both anti- and procoagulant molecules including prostacyclin and thromboxane A_2, respectively. A coordinated balance between platelet inhibition with prostacyclin and platelet activation with thromboxane A_2 is critical to maintain hemostasis and prevent thrombosis. Aspirin remains to be the major treatment to decrease the risk for major adverse cardiac events, including myocardial infarction and stroke [117]. Aspirin is generally used in combination with other antiplatelet medications to achieve a steady antiplatelet effect since aspirin alone would not inhibit other molecular pathways leading to platelet activation. A combination of aspirin with $P2Y_{12}$ or PDE inhibitors has shown to provide great additive and, in some instances, synergistic antiplatelet effect, however, generally at the expense of increased risks of bleeding.

The family of purinergic $P2Y_{12}$ receptor inhibitors includes 5 members: ticlopidine, clopidogrel, prasugrel, ticagrelor, and cangrelor. $P2Y_{12}$ antagonism is the next choice when dual-antiplatelet therapy (e.g., with aspirin) is indicated. Structurally, purinergic receptor inhibitors are related but not similar. Ticlopidine, clopidogrel, and prasugrel are thienopyridines with ticagrelor being a cyclopentyltriazolopyrimidine, whereas cangrelor is a non-hydrolyzable ATP analog. Ticlopidine is associated with severe myelosuppression and therefore not commonly used. The next two members of this group, prasugrel and clopidogrel, require a two-step enzymatic conversion to their active metabolites by the intestinal carboxylesterases and/or hepatic cytochrome P450 system, making their onset of action slow with 2-8 hours after the first dose to achieve an adequate platelet inhibition. It is also worth noting that prasugrel, unlike clopidogrel, is effective in most individuals independent of CYP2C19 polymorphism, which is associated with interindividual variability of clopidogrel conversion to its active pharmacological metabolite. These two members have a slower offset of action due to the irreversible nature of their inhibition of $P2Y_{12}$. And the last two members of this family, ticagrelor and cangrelor are reversible inhibitors of $P2Y_{12}$ that do not require biotransformation in the liver, therefore having faster onset and offset of action. In clinical studies, ticagrelor compared to clopidogrel resulted in significantly better outcomes and decreased mortality in patients with STEMI and NSTEMI. Cangrelor, being used exclusively in periprocedural settings in percutaneous coronary intervention (PCI) due to its sole availability as an injectable form, is associated with reduced stent thrombosis. However, it is important to note that a significant decrease in thrombotic complications offered by ticagrelor and cangrelor comes at the expense of increased risks for major bleedings.

The next class is PAR1 antagonism, represented by a sole clinically used member, vorapaxar. As described above, PAR signaling by thrombin is a potent activation pathway in platelets. PAR1 antagonism has been shown to reduce thrombotic cardiovascular events in myocardial infarction and peripheral vascular disease patients [118]. Vorapaxar competes with the tethered ligand of PAR1 generated by thrombin and prevents signal transduction. A study investigating efficacy and safety of vorapaxar in patients with non-ST elevation acute coronary syndrome demonstrated a signif-

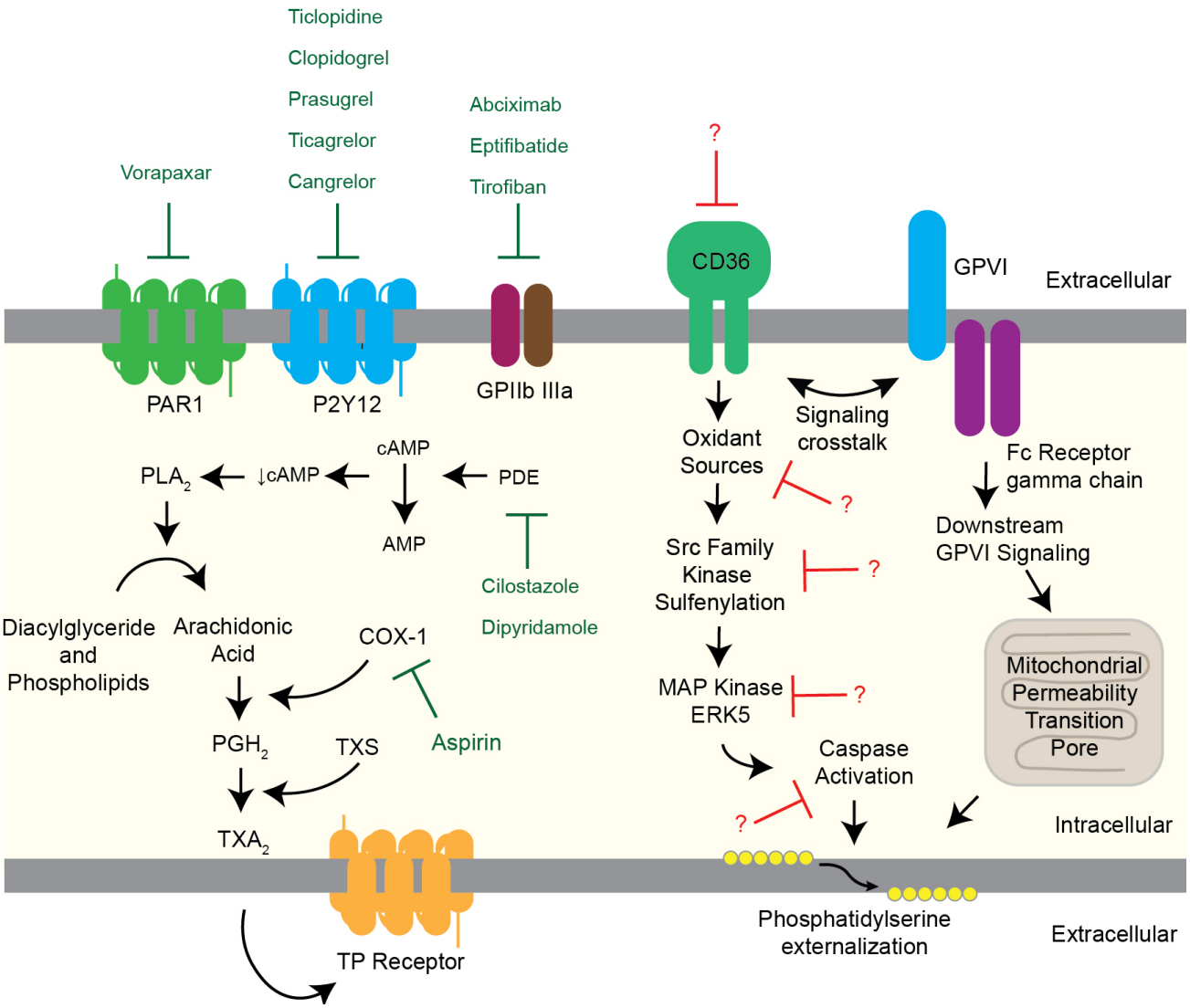

Fig. 3. Potential therapeutic targets for antiplatelet medications. Food and Drug Administration-approved antiplatelet agents are shown in green against varying targets in the platelet activation pathway by classic agonists. Vorapaxar targets the thrombin receptor protease-activated receptor (PAR) 1. Ticlopidine, clopidogrel, prasugrel, ticagrelor, and cangrelor target the ADP receptor $P2Y_{12}$. Integrin $\alpha IIb\beta 3$ (GPIIbIIIa) antagonists, abciximab, eptifibatide, and tirofiban prevent a conformational change of the protein to an activated state that could bind to soluble fibrinogen. Phosphodiesterase antagonists, cilostazol and dipyridamole, prevent cAMP hydrolyzation to AMP, which in turn down-regulates phospholipase A_2 and subsequent arachidonic acid release for thromboxane A_2 synthesis. Lastly, the mainstream cyclooxygenase antagonist is aspirin, which prevents the generation of thromboxane A_2 from its precursors. In the CD36 prothrombotic pathway, CD36 amplifies the GPVI signaling pathway to promote a prothrombotic and procoagulant phenotype. Potential antagonism in the pathway is shown in red.

icantly increased risk of major bleeding, hence this study was terminated early [119]. A separate study has shown that vorapaxar, although decreasing risks of cardiovascular mortality, can lead to major bleedings, including intracranial hemorrhages [120]. Therefore, vorapaxar is used as secondary prevention for myocardial infarction and peripheral artery disease, while being contraindicated in patients with a history of stroke due to higher probability and severity of bleeding complications.

The next class of antiplatelet medications is direct inhibitors of integrin $\alpha IIb\beta 3$, also known as glycoprotein IIbIIIa (GPIIbIIIa). Integrin $\alpha IIb\beta 3$ is the most abundant adhesion receptor on the platelet surface that bridges platelet-platelet interaction through its binding with fibrinogen. All platelet activation pathways lead to integrin $\alpha IIb\beta 3$ activations, and as such integrin $\alpha IIb\beta 3$ is an attractive therapeutic target to prevent thrombosis. Several integrin $\alpha IIb\beta 3$ antagonists are currently used clinically, including tirofiban, eptifibatide, and abciximab. Abciximab is a Fab fragment of the chimeric human-murine monoclonal antibody 7E3 and targets both integrin $\alpha IIb\beta 3$ and $\alpha v\beta 3$ [121, 122]. Abciximab remains in circulation for at least two weeks with the normal recovery of platelet functions within 48 hours [123]. Eptifibatide, a cyclic heptapeptide based on the recognition se-

Table 1. List of clinically used antiplatelet medications.

Name	Mechanism of Action	Clinical (Labelled) Use	Hematologic Adverse Effects
Abciximab	Integrin α2bß3 (glycoprotein IIbIIIa) antagonism	Prevent thrombotic complications in patients with NSTEMI and those undergoing PCI	• Hemorrhage • Thrombocytopenia
Eptifibatide			
Tirofiban			
Ticlopidine	Irreversible inhibition of P2Y12 receptors	Decrease the risk of thrombotic stroke in patients with a history of stroke	• Hemorrhage • Neutropenia • Thrombocytopenia • TTP • Aplastic anemia
Clopidogrel		Decrease the rate of MI and stroke in patients with NSTEMI, including both patients managed pharmacologically or undergoing PCI.	• Hemorrhage • Neutropenia • TTP
Prasugrel		Prevent thrombotic complications in patients undergoing PCI for unstable angina, NSTEMI or STEMI	• Hemorrhage • Leucopenia • Anemia
Ticagrelor	Reversible inhibition of P2Y12	Prevent thrombotic complications in patients undergoing PCI.	• Hemorrhage
Cangrelor			
Vorapaxar	Reversible inhibition of PAR1	Reduce thrombotic complications in patients with a history of MI or PAD	• Hemorrhage
Aspirin	Irreversible inhibition of cyclooxygenase 1	Secondary prevention after acute coronary syndromes, and management of ischemic heart disease.	• Hemorrhage
Cilostazol	Inhibition of cAMP phosphodiesterase 3	Intermittent Claudication	• Thrombocytopenia • Leucopenia
Dipyridamole	Inhibition of phosphodiesterase	Prevent thrombosis post artificial heart valve replacement	• Thrombocytopenia

cAMP, cyclic adenosine monophosphate; MI, myocardial infarction; NSTEMI, non-ST elevation myocardial infarction; $P2Y_{12}$, P2Y purinergic receptor 12; PAD, peripheral artery disease; PAR1, protease-activated receptor 1; PCI, percutaneous coronary intervention; STEMI, ST elevation myocardial infarction; TTP, thrombotic thrombocytopenic purpura.

quence found in snake venom, binds reversibly to integrin αIIbβ3, and has a half-life of 4-5 hours. This class of antiplatelet medications is primarily used in patients undergoing percutaneous coronary intervention. All members of this group can lead to hemorrhage and thrombocytopenia.

And finally, the last group of antiplatelet medications is inhibitors of phosphodiesterases. There are two members of this class that is approved for clinical use in the United States, cilostazol and dipyridamole. Although possibly inhibiting different isotypes of phosphodiesterase the net therapeutic effect of both medications is increased levels of cyclic AMP (cAMP). Elevation of intracellular cAMP induces the activation of protein kinase A (PKA), which in turn increases the threshold for platelet activation. Besides platelet effect, increased levels of cAMP and PKA in vascular smooth muscle cells prevent activation of myosin light-chain kinase that is important in the contraction of smooth muscle cells, thereby exerting its vasodilatory effect. These effects are responsible for the primary use of cilostazol in patients with intermittent claudication.

Like any other pharmacologic agent, antiplatelets can cause adverse effects. An adverse effect is an undesirable secondary effect, which occurs in addition to the primary desired therapeutic effect of medicines. Side effects are patient-variable and are dependent upon the patient's age, gender, ethnicity, weight, as well as general physical health. Side effects of antiplatelet medicines can be diverse. Table 1 outlines the major side effects of clinically used antiplatelet medications but only to the extent of the hematologic system. $P2Y_{12}$ inhibitors together with aspirin are the most used antiplatelets. Although with the evolution of $P2Y_{12}$ antagonism many adverse effects like myelosuppression (seen with ticlopidine), thrombotic thrombocytopenic purpura (seen both with clopidogrel and ticlopidine) are not an issue with the third generation of $P2Y_{12}$ inhibitors like ticagrelor and cangrelor, bleeding remains to be the major adverse effect. The risk for bleeding complications is further increased if the patient is placed on a dual antiplatelet regimen (e.g., aspirin plus $P2Y_{12}$ antagonist). Hemorrhages vary in degree with different antiplatelets, possibly being more severe with PAR1 antagonism and less severe with cyclooxygenase inhibition. But even in mild severity hemorrhages decrease the quality of life. Managing patients on antiplatelet medications is further aggravated by limitations in clinical tools assessing platelet functionalities to make it possible to individually titrate the antiplatelet effect of a pharmaceutical agent to the

point of minimal bleeding risk and greatest antithrombotic benefit. Considering all the challenges there is a need for an antiplatelet approach that would have minimal impact on hemostatic aspects of platelet activation and would rather target cellular/molecular aspects leading to thrombosis. In the light of new knowledge, there are few novel pharmacological targets (Fig. 3) for potential antiplatelet therapy development.

CD36. CD36 is a highly expressed receptor on the surface of platelets. As discussed in previous sections, CD36 is the cornerstone for platelet contribution to thrombosis in dyslipidemic settings. Therefore, CD36 antagonism is the first logical antiplatelet candidate in the face of antithrombotic therapy in dyslipidemia. As shown previously, molecular signaling of CD36 stimulated by oxLDL leads to amplification of the physiologic procoagulant mechanisms downstream of GPVI leading to thrombosis [24]. And therefore, inhibition of CD36 signaling should provide a beneficial antithrombotic effect without affecting platelet hemostatic functionalities. Indeed, it has been shown that in the absence of CD36 oxLDL-stimulated amplification of GPVI-mediated procoagulant response is abrogated [24]. CD36 is also essential in monocyte/macrophage for the progression of atherosclerosis [124]. This indicates that CD36 is an attractive candidate not only for its antithrombotic potential but also to halt the progression of atherosclerosis. However, there are no pharmacodynamic or toxicologic studies to date determining the adverse effects of short- or long-term CD36 inhibition *in vivo*. One potential limitation of CD36 inhibition is the fact that CD36, being a scavenger receptor, is expressed in many cells of the body and binds many different ligands. For instance, CD36 binds cell wall components of the bacteria from *Staphylococcus* and *Mycobacterium* genus, and therefore one potential complication of long-term CD36 antagonism is increased susceptibility to these infectious agents. Another aspect of CD36 functionality comes from the fact that in myocytes and adipocytes CD36 binds long-chain fatty acids (LCFA) to transport them into the cell for beta-oxidation and lipid storage, respectively [58]. And therefore, CD36 antagonism may lead to a decreased metabolic activity of the cells that rely on CD36-mediated LCFA import (e.g. cardiomyocytes, skeletal muscle cells, adipocytes, etc.). This in turn can lead to a variety of adverse effects like cardiotoxicity, including cardiomyopathy and cardiac arrhythmias, rhabdomyolysis, and fasting hypoglycemia. These aspects of CD36 functionalities must be considered in the development of therapeutic CD36 antagonists.

An alternative method is to consider CD36 as a theragnostic approach. Genetic studies on CD36 reported that the *CD36* gene has specific single-nucleotide polymorphism that is linked to major adverse cardiac events with its surface expression levels [73, 125]. More specifically, the platelet surface expression levels on individuals vary considerably (up to 20,000 copies per platelet) and correlate to the reactivity with oxidized lipids [73]. These genetic studies suggest the possibility of using CD36 expression as a predictive marker for potential adverse events in hyperlipidemic individuals. In this case, it could be possible to consider the use of antiplatelet therapy with individuals at high risk for thrombotic events based on their CD36 expression profile. This, however, would require a full spectrum of experimental evidence.

NADPH oxidase. Platelet CD36 was shown to promote oxidant generation by NADPH oxidase in dyslipidemia [71, 126, 127]. These studies outline NADPH oxidase as a possible pharmaceutical target in dyslipidemia-associated thrombotic complications. There have been efforts to develop specific NADPH oxidase inhibitors [27, 128, 129]. However, although platelets express NADPH oxidase, their oxidant generation output is much less than the oxidative burst mechanism induced in immune cells. Therefore, significant efforts would be required to develop a platelet specific NADPH oxidase inhibitor, since non-specific inhibition in white blood cells would result in chronic granulomatous disease-like syndrome with patients being prone to infections caused by catalase-positive organisms. Therefore, NADPH oxidase inhibition is not an attractive candidate for antithrombotic therapy in dyslipidemic settings.

Src kinase. The Src kinase family is a family of nine nonreceptor tyrosine kinases and are attractive targets for inhibition in many diseases. The three most highly expressed members in platelets from this family are Src, Lyn, and Fyn. These members are associated with integrin, GPIb-IX, and ITAM signaling [130]. They also provide a significant contribution to the G_q and G_i downstream signaling [131, 132], whereas CD36 downstream signaling is associated with Fyn, Lyn, and Yes [74]. Yet, selective targeting of Src family kinases has not been achieved in the platelet system nor the context of atherothrombosis because of their expression in multiple cell types in the vasculature. Given the essential role of Src family kinases in platelet signal transduction, Src family kinase antagonism in platelets in the context of atherothrombosis could impair their hemostatic capabilities, and therefore any potential non-specific Src kinase inhibitor can have bleeding adverse effects. This is supported by the fact that easy bruising, hematuria, and melena are among the side effects of imatinib, an FDA-approved Src kinase inhibitor. Another member of the Src kinase inhibitor family, dasatinib inhibits GPVI stimulated procoagulant platelet formation and can lead to thrombocytopenia and bleeding [133, 134]. On the other hand, the evidence that Src kinases are selective targets of specific oxidants and promote their activity suggests that the oxidation mechanism of Src kinases by CD36 signaling could be exploited as a potential node of antagonism [81]. Inhibition of ROS generation should not alter the functionalities of platelets in physiologic hemostasis. This, however, must be demonstrated experimentally.

MAP kinase/ERK5. MAP kinase ERK5 is a member of the MAP kinase family and is present and functional in platelets. MAP kinase ERK5 is different from the other MAP kinases by having a distinct activation pathway via MEKK2/3

and MEK5 [135]. MAP kinase ERK5 is a redox-sensor and regulates platelet activation in conditions with greatly elevated reactive oxygen species generation [86, 87, 126]. Specifically, ERK5 is a redox switch to promote maladaptive platelet signaling during ST-elevated myocardial infarction and in hypoxic conditions [86, 87]. In dyslipidemia, ERK5 is a redox sensor that links CD36 to platelet pro-aggregatory and procoagulant fibrin deposition in dyslipidemia [126]. Selective small molecule inhibitors to the MEK5-ERK5 signaling pathway are available [136]. However, no specific antagonists to MEK5-ERK5 in the platelet system have been achieved likely due to the structural overlap between ERK5 and other MAP kinases [137] and its expression in other cell types including monocytes/macrophages [138] and endothelial cells [139]. Selective antagonism of ERK5 would potentially negate heightened platelet proaggregatory and procoagulant state in dyslipidemia or other conditions associated with greatly elevated redox stress [140]. Nonetheless, long-term platelet ERK5 antagonism in the context of dyslipidemia requires further investigation.

Caspase. Caspase activity triggers a plethora of cellular functions. The signature understanding of caspase activation in platelets is the externalization of PSer for the turnover of aged platelets. In dyslipidemia, oxidized lipid signaling through CD36 and ERK5 promotes caspase activity promoting PSer externalization for a procoagulant phenotype [24]. In this context, the small molecular caspase inhibitor z-VAD-fmk along with the genetic deletion of the regulators of apoptosis, Bak and Bax, prevented CD36 and ERK5 sensitization of PSer externalization by GPVI [24]. Also, genetic deletion of CD36 and ERK5 prevented the procoagulant fibrin deposition by caspases *in vivo,* suggesting inhibition of caspases downstream of CD36/ERK5 in this setting could potentially maintain the hemostatic capabilities by the GPVI pathway. Caspases also play a major role in megakaryocyte apoptosis required for platelet formation. Together with the fact that caspases are important in platelet clearance, long-term antagonism of caspases *in vivo* may result in platelet aging within the circulation. This in turn will present as a bleeding phenotype knowing aged platelets are associated with longer bleeding times due to decreased platelet adhesiveness. Therefore, caspase inhibition is not an attractive approach for antiplatelet therapy.

CD36-independent pathways. In the last decade, several studies suggest anti-atherosclerotic and antithrombotic effects of selective lipids that may indirectly counterbalance the CD36 prothrombotic pathway. Omega-3 fatty acids are a type of polyunsaturated fatty acids with antioxidative properties and have been shown to have health benefits in many systemic inflammatory conditions, including thrombosis [141]. Omega-3 fatty acids were shown to decrease the surface expression levels of CD36 on different cell types including monocytes/macrophages [142], adipose cells [143], endothelial cells [144], and cardiomyocytes [145]. In the platelet system, omega-3 polyunsaturated fatty acids and their downstream oxylipin metabolites decrease platelet activation, adhesion, and arterial thrombosis in murine models [146]. However, whether omega-3 fatty acids impact CD36 expression or signaling in platelets is unclear. Despite this fact, clinical trials suggest a therapeutic benefit of omega-3 fatty acids in the context of adverse cardiac events [147, 148]. Of recent, the REDUCE-IT trial shows therapeutic benefits in primary and secondary outcomes when daily 4 g of icosapent ethyl, a stable omega-3 fatty acid derivative, was administered to patients receiving statin therapy [147, 149]. This study suggest CD36-independent pathways for preventing atherothrombosis that warrants mechanistic investigation.

In conclusion, although there are numerous FDA-approved antiplatelet medications, their current utility is limited due to the risk of bleeding complications. Platelet activation promotes either a pro-aggregatory or procoagulant state that is agonist dependent. Specifically, the collagen receptor GPVI and the PAR thrombin receptors are the most potent activation pathways in platelets. In dyslipidemia, specific signaling pathways have been in the spotlight, including the prothrombotic scavenger receptor CD36 signaling that enhances platelet pro-aggregatory and procoagulant functions through sensitization of GPVI for procoagulant PSer externalization and fibrin deposition. In addition to the CD36 prothrombotic phenotype, platelet reactivity in dyslipidemia may also be in part due to the sensitivity of the bone marrow niche to cholesterol. Further identification of specific signaling nodes in the CD36 pathway that is distinct from classic platelet activation would potentially identify novel antiplatelet targets in dyslipidemia and beyond.

Author contributions

MY and AK wrote and edited the manuscript.

Acknowledgment

The authors declare no acknowledgment.

Funding

M.Y. is supported by a National Institute of Health (NIH) National Heart Lung and Blood Institute T32 training grant T32HL007917.

Conflict of interest

The authors declare no competing conflict of interest.

References

[1] Wendelboe AM, Raskob GE. Global burden of thrombosis. Circulation Research. 2016; 118: 1340-1347.

[2] Bonaca MP, Bhatt DL, Steg PG, Storey RF, Cohen M, Im K, *et al.* Ischaemic risk and efficacy of ticagrelor in relation to time from P2Y12 inhibitor withdrawal in patients with prior myocardial infarction: insights from PEGASUS-TIMI 54. European Heart Journal. 2016; 37: 1133-1142.

[3] Scirica BM, Bonaca MP, Braunwald E, De Ferrari GM, Isaza D, Lewis BS, *et al.* Vorapaxar for secondary prevention of thrombotic events for patients with previous myocardial infarction: a prespecified subgroup analysis of the TRA 2°P-TIMI 50 trial. The Lancet. 2012; 380: 1317-1324.

[4] Rai V, Balters MW, Agrawal DK. Factors IX, XI, and XII: potential therapeutic targets for anticoagulant therapy in atherothrombosis. Reviews in Cardiovascular Medicine. 2019; 20: 245-253.
[5] Ducrocq G, Wallace JS, Baron G, Ravaud P, Alberts MJ, Wilson PWF, *et al.* Risk score to predict serious bleeding in stable outpatients with or at risk of atherothrombosis. European Heart Journal. 2010; 31: 1257-1265.
[6] Nelson RH. Hyperlipidemia as a risk factor for cardiovascular disease. Primary Care: Clinics in Office Practice. 2013; 40: 195-211.
[7] Podrez EA, Byzova TV, Febbraio M, Salomon RG, Ma Y, Valiyaveettil M, *et al.* Platelet CD36 links hyperlipidemia, oxidant stress and a prothrombotic phenotype. Nature Medicine. 2007; 13: 1086-1095.
[8] Betteridge DJ, Cooper MB, Saggerson ED, Prichard BN, Tan KC, Ling E, *et al.* Platelet function in patients with hypercholesterolaemia. European Journal of Clinical Investigation. 1994; 24: 30-33.
[9] Carvalho ACA, Colman RW, Lees RS. Platelet function in hyperlipoproteinemia. New England Journal of Medicine. 1974; 290: 434-438.
[10] Stuart MJ, Gerrard JM, White JG. Effect of cholesterol on production of thromboxane B2 by platelets *in vitro*. New England Journal of Medicine. 1980; 302: 6-10.
[11] Stewart LK, Kline JA. Metabolic syndrome increases risk of venous thromboembolism recurrence after acute deep vein thrombosis. Blood Advances. 2020; 4: 127-135.
[12] Wang N, Tall AR. Cholesterol in platelet biogenesis and activation. Blood. 2016; 127: 1949-1953.
[13] Ichinohe T, Takayama H, Ezumi Y, Arai M, Yamamoto N, Takahashi H, *et al.* Collagen-stimulated activation of Syk but not c-Src is severely compromised in human platelets lacking membrane glycoprotein VI. Journal of Biological Chemistry. 1997; 272: 63-68.
[14] Yanaga F, Poole A, Asselin J, Blake R, Schieven GL, Clark EA, *et al.* Syk interacts with tyrosine-phosphorylated proteins in human platelets activated by collagen and cross-linking of the Fc γ-IIA receptor. Biochemical Journal. 1995; 311: 471-478.
[15] Rayes J, Watson SP, Nieswandt B. Functional significance of the platelet immune receptors GPVI and CLEC-2. Journal of Clinical Investigation. 2019; 129: 12-23.
[16] Kholmukhamedov A, Janecke R, Choo H-, Jobe SM. The mitochondrial calcium uniporter regulates procoagulant platelet formation. Journal of Thrombosis and Haemostasis. 2018; 16: 2315-2321.
[17] Rozenvayn N, Flaumenhaft R. Phosphatidylinositol 4,5-bisphosphate mediates Ca^{2+}-induced platelet α-granule secretion. Journal of Biological Chemistry. 2001; 276: 22410-22419.
[18] Nagy M, Perrella G, Dalby A, Becerra MF, Garcia Quintanilla L, Pike JA, *et al.* Flow studies on human GPVI-deficient blood under coagulating and noncoagulating conditions. Blood Advances. 2020; 4: 2953-2961.
[19] Ahmed MU, Kaneva V, Loyau S, Nechipurenko D, Receveur N, Le Bris M, *et al.* Pharmacological blockade of GPVI promotes thrombus disaggregation in the absence of thrombin. Arteriosclerosis, Thrombosis, and Vascular Biology. 2020; 40: 2127-2142.
[20] Akbar H, Duan X, Piatt R, Saleem S, Davis AK, Tandon NN, *et al.* Small molecule targeting the Rac1-NOX2 interaction prevents collagen-related peptide and thrombin-induced reactive oxygen species generation and platelet activation. Journal of Thrombosis and Haemostasis. 2018; 16: 2083-2096.
[21] Walsh TG, Berndt MC, Carrim N, Cowman J, Kenny D, Metharom P. The role of Nox1 and Nox2 in GPVI-dependent platelet activation and thrombus formation. Redox Biology. 2014; 2: 178-186.
[22] Delaney MK, Kim K, Estevez B, Xu Z, Stojanovic-Terpo A, Shen B, *et al.* Differential roles of the NADPH-oxidase 1 and 2 in platelet activation and thrombosis. Arteriosclerosis, Thrombosis, and Vascular Biology. 2016; 36: 846-854.
[23] Choo H, Saafir TB, Mkumba L, Wagner MB, Jobe SM. Mitochondrial calcium and reactive oxygen species regulate agonist-initiated platelet phosphatidylserine exposure. Arteriosclerosis, Thrombosis, and Vascular Biology. 2012; 32: 2946-2955.
[24] Yang M, Kholmukhamedov A, Schulte ML, Cooley BC, Scoggins NO, Wood JP, *et al.* Platelet CD36 signaling through ERK5 promotes caspase-dependent procoagulant activity and fibrin deposition *in vivo*. Blood Advances. 2018; 2: 2848-2861.
[25] Sonkar VK, Kumar R, Jensen M, Wagner BA, Sharathkumar AA, Miller FJ, *et al.* Nox2 NADPH oxidase is dispensable for platelet activation or arterial thrombosis in mice. Blood Advances. 2019; 3: 1272-1284.
[26] Vara D, Mailer RK, Tarafdar A, Wolska N, Heestermans M, Konrath S, *et al.* NADPH oxidases are required for full platelet activation *in vitro* and thrombosis *in vivo* but dispensable for plasma coagulation and hemostasis. Arteriosclerosis, Thrombosis, and Vascular Biology. 2020; ATVBAHA120315565.
[27] Vara D, Tarafdar A, Celikag M, Patinha D, Gulacsy CE, Hounslea E, *et al.* NADPH oxidase 1 is a novel pharmacological target for the development of an antiplatelet drug without bleeding side effects. the FASEB Journal. 2020; 34: 13959-13977.
[28] Wang X, Zhang S, Ding Y, Tong H, Xu X, Wei G, *et al.* P47phox deficiency impairs platelet function and protects mice against arterial and venous thrombosis. Redox Biology. 2020; 34: 101569.
[29] Nieman MT. Protease-activated receptors in hemostasis. Blood. 2016; 128: 169-177.
[30] Han X, Nieman MT. PAR4 (protease-activated receptor 4): PARticularly important 4 antiplatelet therapy. Arteriosclerosis, Thrombosis, and Vascular Biology. 2018; 38: 287-289.
[31] Arachiche A, Mumaw MM, de la Fuente M, Nieman MT. Protease-activated receptor 1 (PAR1) and PAR4 heterodimers are required for PAR1-enhanced cleavage of PAR4 by α-thrombin. Journal of Biological Chemistry. 2013; 288: 32553-32562.
[32] Swords NA, Mann KG. The assembly of the prothrombinase complex on adherent platelets. Arteriosclerosis and Thrombosis: a Journal of Vascular Biology. 1993; 13: 1602-1612.
[33] Sims PJ, Wiedmer T, Esmon CT, Weiss HJ, Shattil SJ. Assembly of the platelet prothrombinase complex is linked to vesiculation of the platelet plasma membrane. Journal of Biological Chemistry. 1989; 264: 17049-17057.
[34] Yang M, Silverstein RL. CD36 and ERK5 link dyslipidemia to apoptotic-like platelet procoagulant function. Current Opinion in Hematology. 2019; 26: 357-365.
[35] Reddy EC, Rand ML. Procoagulant phosphatidylserine-exposing platelets *in vitro* and *in vivo*. Frontiers Cardiovascular Medicine. 2020;7: 15.
[36] Hua VM, Chen VMY. Procoagulant platelets and the pathways leading to cell death. Seminars in Thrombosis and Hemostasis. 2015; 41: 405-412.
[37] Kholmukhamedov A. Procoagulant Platelets. In: Platelets. IntechOpen. 2020.
[38] Brunner JD, Schenck S, Dutzler R. Structural basis for phospholipid scrambling in the TMEM16 family. Current Opinion in Structural Biology. 2016; 39: 61-70.
[39] van Kruchten R, Mattheij NJA, Saunders C, Feijge MAH, Swieringa F, Wolfs JLN, *et al.* Both TMEM16F-dependent and TMEM16F-independent pathways contribute to phosphatidylserine exposure in platelet apoptosis and platelet activation. Blood. 2013; 121: 1850-1857.
[40] Falzone ME, Rheinberger J, Lee BC, Peyear T, Sasset L, Raczkowski AM, *et al.* Structural basis of Ca^{2+}-dependent activation and lipid transport by a TMEM16 scramblase. Elife. 2019; 8: e43229.
[41] Jobe SM, Wilson KM, Leo L, Raimondi A, Molkentin JD, Lentz SR, *et al.* Critical role for the mitochondrial permeability transition pore and cyclophilin D in platelet activation and thrombosis. Blood. 2008; 111: 1257-1265.

[42] Baig AA, Haining EJ, Geuss E, Beck S, Swieringa F, Wanitchakool P, *et al.* TMEM16F-mediated platelet membrane phospholipid scrambling is critical for hemostasis and thrombosis but not thromboinflammation in mice-brief report. Arteriosclerosis, Thrombosis, and Vascular Biology. 2016; 36: 2152-2157.
[43] Suzuki J, Umeda M, Sims PJ, Nagata S. Calcium-dependent phospholipid scrambling by TMEM16F. Nature. 2010; 468: 834-838.
[44] Schoenwaelder SM, Yuan Y, Josefsson EC, White MJ, Yao Y, Mason KD, *et al.* Two distinct pathways regulate platelet phosphatidylserine exposure and procoagulant function. Blood. 2009; 114: 663-666.
[45] Podoplelova NA, Sveshnikova AN, Kotova YN, Eckly A, Receveur N, Nechipurenko DY, *et al.* Coagulation factors bound to procoagulant platelets concentrate in cap structures to promote clotting. Blood. 2016; 128: 1745-1755.
[46] Kirkpatrick AC, Vincent AS, Dale GL, Prodan CI. Increased platelet procoagulant potential predicts recurrent stroke and TIA after lacunar infarction. Journal of Thrombosis and Haemostasis. 2020; 18: 660-668.
[47] Alberio L, Safa O, Clemetson KJ, Esmon CT, Dale GL. Surface expression and functional characterization of α-granule factor V in human platelets: effects of ionophore a23187, thrombin, collagen, and convulxin. Blood. 2000; 95: 1694-1702.
[48] Jobe SM, Leo L, Eastvold JS, Dickneite G, Ratliff TL, Lentz SR, *et al.* Role of FcRγ and factor XIIIA in coated platelet formation. Blood. 2005; 106: 4146-4151.
[49] Debreceni IB, Mezei G, Batár P, Illés Á, Kappelmayer J. Dasatinib inhibits procoagulant and clot retracting activities of human platelets. International Journal of Molecular Sciences. 2019; 20: 5430.
[50] Munnix I, Harmsma M, Giddings J, Collins P, Feijge M, Comfurius P, *et al.* Store-mediated calcium entry in the regulation of phosphatidylserine exposure in blood cells from Scott patients. Thrombosis and Haemostasis. 2003; 89: 687-695.
[51] Gilio K, van Kruchten R, Braun A, Berna-Erro A, Feijge MAH, Stegner D, *et al.* Roles of platelet STIM1 and Orai1 in glycoprotein VI- and thrombin-dependent procoagulant activity and thrombus formation. Journal of Biological Chemistry. 2010; 285: 23629-23638.
[52] Wang Y, Han Y, She J, Nguyen NX, Mootha VK, Bai XC, *et al.* Structural insights into the Ca $^{2+}$-dependent gating of the human mitochondrial calcium uniporter. Elife. 2020; 9: e60513.
[53] Wang C, Jacewicz A, Delgado BD, Baradaran R, Barstow Long S. Structures reveal gatekeeping of the mitochondrial Ca^{2+} uniporter by MICU1-MICU2. Elife. 2020; 9: e59991.
[54] Fan M, Zhang J, Tsai C, Orlando BJ, Rodriguez M, Xu Y, *et al.* Structure and mechanism of the mitochondrial Ca^{2+} uniporter holocomplex. Nature. 2020; 582: 129-133.
[55] Rimessi A, Pozzato C, Carparelli L, Rossi A, Ranucci S, De Fino I, *et al.* Pharmacological modulation of mitochondrial calcium uniporter controls lung inflammation in cystic fibrosis. Science Advances. 2020; 6: eaax9093.
[56] Levitan I, Volkov S, Subbaiah PV. Oxidized LDL: diversity, patterns of recognition, and pathophysiology. Antioxidants & Redox Signaling. 2010; 13: 39-75.
[57] Clemetson KJ, Pfueller SL, Luscher EF, Jenkins CSP. Isolation of the membrane glycoproteins of human blood platelets by lectin affinity chromatography. Biochimica et Biophysica Acta - Biomembranes. 1977; 464: 493-508.
[58] Coburn CT, Knapp FF, Febbraio M, Beets AL, Silverstein RL, Abumrad NA. Defective uptake and utilization of long chain fatty acids in muscle and adipose tissues of CD36 knockout mice. Journal of Biological Chemistry. 2000; 275: 32523-32529.
[59] Neculai D, Schwake M, Ravichandran M, Zunke F, Collins RF, Peters J, *et al.* Structure of LIMP-2 provides functional insights with implications for SR-BI and CD36. Nature. 2013; 504: 172-176.
[60] Hoosdally SJ, Andress EJ, Wooding C, Martin CA, Linton KJ. The human scavenger receptor CD36. Journal of Biological Chemistry. 2009; 284: 16277-16288.
[61] Wang Y, Fang C, Gao H, Bilodeau ML, Zhang Z, Croce K, *et al.* Platelet-derived myeloid related protein-14 (S100A9) regulates thrombus formation. Journal of Clinical Investigation. 2014; 124: 2160-2171.
[62] Klenotic PA, Page RC, Li W, Amick J, Misra S, Silverstein RL. Molecular basis of antiangiogenic thrombospondin-1 type 1 repeat domain interactions with CD36. Arteriosclerosis, Thrombosis, and Vascular Biology. 2013; 33: 1655-1662.
[63] Zhu W, Li W, Silverstein RL. Advanced glycation end products induce a prothrombotic phenotype in mice via interaction with platelet CD36. Blood. 2012; 119: 6136-6144.
[64] Ghosh A, Li W, Febbraio M, Espinola RG, McCrae KR, Cockrell E, *et al.* Platelet CD36 mediates interactions with endothelial cell - derived microparticles and contributes to thrombosis in mice. Journal of Clinical Investigation. 2008; 118: 1934-1943.
[65] Guthmann F, Maehl P, Preiss J, Kolleck I, Rüstow B. Ectoprotein kinase-mediated phosphorylation of FATCD36 regulates palmitate uptake by human platelets. Cellular and Molecular Life Sciences. 2002; 59: 1999-2003.
[66] Calvo D, Gómez-coronado D, Suárez Y, Lasunción MA, Vega MA. Human CD36 is a high affinity receptor for the native lipoproteins HDL, LDL, and VLDL. Journal of Lipid Research. 1998; 39: 777-788.
[67] Nilsen NJ, Deininger S, Nonstad U, Skjeldal F, Husebye H, Rodionov D, *et al.* Cellular trafficking of lipoteichoic acid and Toll-like receptor 2 in relation to signaling; role of CD14 and CD36. Journal of Leukocyte Biology. 2008; 84: 280-291.
[68] Yang M, Silverstein RL. CD36 signaling in vascular redox stress. Free Radical Biology and Medicine. 2019; 136: 159-171.
[69] Roberts W, Magwenzi S, Aburima A, Naseem KM. Thrombospondin-1 induces platelet activation through CD36-dependent inhibition of the cAMP/protein kinase a signaling cascade. Blood. 2010; 116: 4297-4306.
[70] Berger M, Raslan Z, Aburima A, Magwenzi S, Wraith KS, Spurgeon BEJ, *et al.* Atherogenic lipid stress induces platelet hyperactivity through CD36-mediated hyposensitivity to prostacyclin: the role of phosphodiesterase 3a. Haematologica. 2020; 105: 808-819.
[71] Magwenzi S, Woodward C, Wraith KS, Aburima A, Raslan Z, Jones H, *et al.* Oxidized LDL activates blood platelets through CD36/NOX2–mediated inhibition of the cGMP/protein kinase G signaling cascade. Blood. 2015; 125: 2693-2703.
[72] Moebius J, Zahedi RP, Lewandrowski U, Berger C, Walter U, Sickmann A. The human platelet membrane proteome reveals several new potential membrane proteins. Molecular & Cellular Proteomics. 2005; 4: 1754-1761.
[73] Ghosh A, Murugesan G, Chen K, Zhang L, Wang Q, Febbraio M, *et al.* Platelet CD36 surface expression levels affect functional responses to oxidized LDL and are associated with inheritance of specific genetic polymorphisms. Blood. 2011; 117: 6355-6366.
[74] Huang MM, Bolen JB, Barnwell JW, Shattil SJ, Brugge JS. Membrane glycoprotein IV (CD36) is physically associated with the Fyn, Lyn, and yes protein-tyrosine kinases in human platelets. Proceedings of the National Academy of Sciences. 1991; 88: 7844-7848.
[75] Ramakrishnan DP, Hajj-Ali RA, Chen Y, Silverstein RL. Extracellular vesicles activate a CD36-dependent signaling pathway to inhibit microvascular endothelial cell migration and tube formation. Arteriosclerosis, Thrombosis, and Vascular Biology. 2016; 36: 534-544.
[76] Chen Y, Kennedy DJ, Ramakrishnan DP, Yang M, Huang W, Li Z, *et al.* Oxidized LDL-bound CD36 recruits an Na^+/K^+-ATPase-Lyn complex in macrophages that promotes atherosclerosis. Science Signaling. 2015; 8: ra91-ra91.
[77] Chen K, Febbraio M, Li W, Silverstein RL. A specific CD36-dependent signaling pathway is required for platelet activation by oxidized low-density lipoprotein. Circulation Research. 2008; 102: 1512-1519.

[78] Chen K, Li W, Major J, Rahaman SO, Febbraio M, Silverstein RL. Vav guanine nucleotide exchange factors link hyperlipidemia and a prothrombotic state. Blood. 2011; 117: 5744-5750.
[79] Yang M, Cooley BC, Li W, Chen Y, Vasquez-Vivar J, Scoggins NO, *et al.* Platelet CD36 promotes thrombosis by activating redox sensor ERK5 in hyperlipidemic conditions. Blood. 2017; 129: 2917-2927.
[80] Wraith KS, Magwenzi S, Aburima A, Wen Y, Leake D, Naseem KM. Oxidized low-density lipoproteins induce rapid platelet activation and shape change through tyrosine kinase and Rho kinase-signaling pathways. Blood. 2013; 122: 580-589.
[81] Yang M, Li W, Harberg C, Chen W, Yue H, Ferreira RB, *et al.* Cysteine sulfenylation by CD36 signaling promotes arterial thrombosis in dyslipidemia. Blood Advances. 2020; 4: 4494-4507.
[82] Heppner DE, Dustin CM, Liao C, Hristova M, Veith C, Little AC, *et al.* Direct cysteine sulfenylation drives activation of the Src kinase. Nature Communications. 2018; 9: 4522.
[83] Gupta V, Carroll KS. Profiling the reactivity of cyclic C-nucleophiles towards electrophilic sulfur in cysteine sulfenic acid. Chemical Science. 2016; 7: 400-415.
[84] Gupta V, Yang J, Liebler DC, Carroll KS. Diverse redoxome reactivity profiles of carbon nucleophiles. Journal of the American Chemical Society. 2017; 139: 5588-5595.
[85] Patel P, Naik UP. Platelet MAPKs-a 20+ year history: what do we really know? Journal of Thrombosis and Haemostasis. 2020; 18: 2087-2102.
[86] Cameron SJ, Ture SK, Mickelsen D, Chakrabarti E, Modjeski KL, McNitt S, *et al.* Platelet extracellular regulated protein kinase 5 is a redox switch and triggers maladaptive platelet responses and myocardial infarct expansion. Circulation. 2015; 132: 47-58.
[87] Cameron SJ, Mix DS, Ture SK, Schmidt RA, Mohan A, Pariser D, *et al.* Hypoxia and ischemia promote a maladaptive platelet phenotype. Arteriosclerosis, Thrombosis, and Vascular Biology. 2018; 38: 1594-1606.
[88] Choo H, Kholmukhamedov A, Zhou C, Jobe S. Inner mitochondrial membrane disruption links apoptotic and agonist-initiated phosphatidylserine externalization in platelets. Arteriosclerosis, Thrombosis, and Vascular Biology. 2017; 37: 1503-1512.
[89] Mason KD, Carpinelli MR, Fletcher JI, Collinge JE, Hilton AA, Ellis S, *et al.* Programmed anuclear cell death delimits platelet life span. Cell. 2007; 128: 1173-1186.
[90] Zhao L, Liu J, He C, Yan R, Zhou K, Cui Q, *et al.* Protein kinase a determines platelet life span and survival by regulating apoptosis. Journal of Clinical Investigation. 2017; 127: 4338-4351.
[91] Aoki I, Aoki N, Kawano K, Shimoyama K, Maki A, Homori M, *et al.* Platelet-dependent thrombin generation in patients with hyperlipidemia. Journal of the American College of Cardiology. 1997; 30: 91-96.
[92] Döhrmann M, Makhoul S, Gross K, Krause M, Pillitteri D, Auer C, *et al.* CD36-fibrin interaction propagates FXI-dependent thrombin generation of human platelets. The FASEB Journal. 2020; 34: 9337-9357.
[93] Aburima A, Berger M, Spurgeon BE, Webb BA, Wraith KS, Febbraio M, *et al.* Thrombospondin-1 promotes haemostasis through modulation of cAMP signalling in blood platelets. Blood. 2020; blood.2020005382.
[94] Asch AS, Barnwell J, Silverstein RL, Nachman RL. Isolation of the thrombospondin membrane receptor. Journal of Clinical Investigation. 1987; 79: 1054-1061.
[95] Nergiz-unal R, Lamers MME, Van Kruchten R, Luiken JJ, Cosemans JMEM, Glatz JFC, *et al.* Signaling role of CD36 in platelet activation and thrombus formation on immobilized thrombospondin or oxidized low-density lipoprotein. Journal of Thrombosis and Haemostasis. 2011; 9: 1835-1846.
[96] Korporaal SJA, Van Eck M, Adelmeijer J, Ijsseldijk M, Out R, Lisman T, *et al.* Platelet activation by oxidized low density lipoprotein is mediated by CD36 and scavenger receptor-a. Arteriosclerosis, Thrombosis, and Vascular Biology. 2007; 27: 2476-2483.
[97] Biswas S, Xin L, Panigrahi S, Zimman A, Wang H, Yakubenko VP, *et al.* Novel phosphatidylethanolamine derivatives accumulate in circulation in hyperlipidemic ApoE−/− mice and activate platelets via TLR2. Blood. 2016; 127: 2618-2629.
[98] Biswas S, Zimman A, Gao D, Byzova TV, Podrez EA. TLR2 plays a key role in platelet hyperreactivity and accelerated thrombosis associated with hyperlipidemia. Circulation Research. 2017; 121: 951-962.
[99] Bartley T. Identification and cloning of a megakaryocyte growth and development factor that is a ligand for the cytokine receptor MpI. Cell. 1994; 77: 1117-1124.
[100] de Sauvage FJ, Hass PE, Spencer SD, Malloy BE, Gurney AL, Spencer SA, *et al.* Stimulation of megakaryocytopoiesis and thrombopoiesis by the c-Mpl ligand. Nature. 1994; 369: 533-538.
[101] Drachman JG, Kaushansky K. Dissecting the thrombopoietin receptor: Functional elements of the Mpl cytoplasmic domain. Proceedings of the National Academy of Sciences. 1997; 94: 2350-2355.
[102] Drachman JG, Griffin JD, Kaushansky K. The c-Mpl ligand (thrombopoietin) stimulates tyrosine phosphorylation of Jak2, Shc, and c-Mpl. Journal of Biological Chemistry. 1995; 270: 4979-4982.
[103] Bacon CM, Justin Tortolani P, Shimosaka A, Rees RC, Longo DL, O'Shea JJ. Thrombopoietin (TPO) induces tyrosine phosphorylation and activation of STAT5 and STAT3. FEBS Letters. 1995; 370: 63-68.
[104] Cramer EM, Norol F, Guichard J, Breton-Gorius J, Vainchenker W, Massé J, e*t al.* Ultrastructure of platelet formation by human megakaryocytes cultured with the Mpl ligand. Blood. 1997; 89: 2336-2346.
[105] Choi E, Nichol J, Hokom M, Hornkohl A, Hunt P. Platelets generated in vitro from proplatelet-displaying human megakaryocytes are functional. Blood. 1995; 85: 402-413.
[106] Grozovsky R, Begonja AJ, Liu K, Visner G, Hartwig JH, Falet H, *et al.* The Ashwell-Morell receptor regulates hepatic thrombopoietin production via JAK2-STAT3 signaling. Nature Medicine. 2015; 21: 47-54.
[107] Harker LA, Hazzard W. Platelet kinetic studies in patients with hyperlipoproteinemia: effects of clofibrate therapy. Circulation. 1979; 60: 492-496.
[108] Pathansali R, Smith N, Bath P. Altered megakaryocyte-platelet haemostatic axis in hypercholesterolaemia. Platelets. 2001; 12: 292-297.
[109] Icli A, Aksoy F, Nar G, Kaymaz H, Alpay MF, Nar R, *et al.* Increased mean platelet volume in familial hypercholesterolemia. Angiology. 2016; 67: 146-150.
[110] Gomes AL, Carvalho T, Serpa J, Torre C, Dias S. Hypercholesterolemia promotes bone marrow cell mobilization by perturbing the SDF-1:CXCR4 axis. Blood. 2010; 115: 3886-3894.
[111] Murphy AJ, Bijl N, Yvan-Charvet L, Welch CB, Bhagwat N, Reheman A, *et al.* Cholesterol efflux in megakaryocyte progenitors suppresses platelet production and thrombocytosis. Nature Medicine. 2013; 19: 586-594.
[112] Kraakman MJ, Lee MKS, Al-Sharea A, Dragoljevic D, Barrett TJ, Montenont E, *et al.* Neutrophil-derived S100 calcium-binding proteins a8/a9 promote reticulated thrombocytosis and atherogenesis in diabetes. Journal of Clinical Investigation. 2017; 127: 2133-2147.
[113] Benlachgar N, Doghmi K, Masrar A, Mahtat EM, Harmouche H, Tazi Mezalek Z. Immature platelets: a review of the available evidence. Thrombosis Research. 2020; 195: 43-50.
[114] Verdoia M, Nardin M, Negro F, Rolla R, De Luca G. Impact of statin therapy on the immature platelet count in patients with coronary artery disease: a single centre cohort study. International Journal of Cardiology. 2018; 272: 40-44.
[115] Patrono C, Morais J, Baigent C, Collet J, Fitzgerald D, Halvorsen S, *et al.* Antiplatelet agents for the treatment and prevention of coronary atherothrombosis. Journal of the American College of Cardiology. 2017; 70: 1760-1776.

[116] Patrono C, Baigent C. Role of aspirin in primary prevention of cardiovascular disease. Nature Reviews Cardiology. 2019; 16: 675-686.
[117] Raber I, McCarthy CP, Vaduganathan M, Bhatt DL, Wood DA, Cleland JGF, *et al.* The rise and fall of aspirin in the primary prevention of cardiovascular disease. the Lancet. 2019; 393: 2155-2167.
[118] Moon JY, Franchi F, Rollini F, Angiolillo DJ. Role for thrombin receptor antagonism with vorapaxar in secondary prevention of atherothrombotic events: from bench to bedside. Journal of Cardiovascular Pharmacology and Therapeutics. 2018; 23: 23-37.
[119] Tricoci P, Huang Z, Held C, Moliterno DJ, Armstrong PW, Van de Werf F, *et al.* Thrombin-receptor antagonist vorapaxar in acute coronary syndromes. New England Journal of Medicine. 2012; 366: 20-33.
[120] Morrow DA, Braunwald E, Bonaca MP, Ameriso SF, Dalby AJ, Fish MP, *et al.* Vorapaxar in the secondary prevention of atherothrombotic events. New England Journal of Medicine. 2012; 366: 1404-1413.
[121] Coller BS, Peerschke EI, Scudder LE, Sullivan CA. A murine monoclonal antibody that completely blocks the binding of fibrinogen to platelets produces a thrombasthenic-like state in normal platelets and binds to glycoproteins IIb and/or IIIa. Journal of Clinical Investigation. 1983; 72: 325-338.
[122] Coller BS. A new murine monoclonal antibody reports on activation-dependent change in the conformation and/or microenvironment of the platelet glycoprotein IIb/IIIa complex. Journal of Clinical Investigation. 1985; 76: 101-108.
[123] Ostrowska M, Adamski P, Koziński M, Navarese EP, Fabiszak T, Grześk G, *et al.* Off-target effects of glycoprotein IIb/IIIa receptor inhibitors. Cardiology Journal. 2014; 21: 458-464.
[124] Silverstein RL, Li W, Park YM, Rahaman SO. Mechanisms of cell signaling by the scavenger receptor CD36: implications in atherosclerosis and thrombosis. Transactions of the American Clinical and Climatological Association. 2010; 121: 206-220.
[125] Love-Gregory L, Sherva R, Schappe T, Qi J, McCrea J, Klein S, *et al.* Common CD36 SNPs reduce protein expression and may contribute to a protective atherogenic profile. Human Molecular Genetics. 2011; 20: 193-201.
[126] Yang M, Cooley BC, Li W, Chen Y, Vasquez-Vivar J, Scoggins NO, *et al.* Platelet CD36 promotes thrombosis by activating redox sensor ERK5 in hyperlipidemic conditions. Blood. 2017; 129: 2917-2927.
[127] Berger M, Wraith K, Woodward C, Aburima A, Raslan Z, Hindle MS, *et al.* Dyslipidemia-associated atherogenic oxidized lipids induce platelet hyperactivity through phospholipase Cγ2-dependent reactive oxygen species generation. Platelets. 2019; 30: 467-472.
[128] Zielonka J, Cheng G, Zielonka M, Ganesh T, Sun A, Joseph J, *et al.* High-throughput assays for superoxide and hydrogen peroxide. Journal of Biological Chemistry. 2014; 289: 16176-16189.
[129] Zielonka J, Zielonka M, Cheng G, Hardy M, Kalyanaraman B. High-throughput screening of NOX inhibitors. Methods in Molecular Biology. 2019; 4: 429-446.
[130] Senis YA, Mazharian A, Mori J. Src family kinases: at the forefront of platelet activation. Blood. 2014; 124: 2013-2024.
[131] Xiang B, Zhang G, Stefanini L, Bergmeier W, Gartner TK, Whiteheart SW, *et al.* The Src family kinases and protein kinase C synergize to mediate Gq-dependent platelet activation. Journal of Biological Chemistry. 2012; 287: 41277-41287.
[132] Kim S, Kunapuli SP. Negative regulation of Gq-mediated pathways in platelets by G12/13 pathways through Fyn kinase. Journal of Biological Chemistry. 2011; 286: 24170-24179.
[133] Quintás-Cardama A, Kantarjian H, Ravandi F, O'Brien S, Thomas D, Vidal-Senmache G, *et al.* Bleeding diathesis in patients with chronic myelogenous leukemia receiving dasatinib therapy. Cancer. 2009; 115: 2482-2490.
[134] Steegmann JL, Baccarani M, Breccia M, Casado LF, García-Gutiérrez V, Hochhaus A, *et al.* European LeukemiaNet recommendations for the management and avoidance of adverse events of treatment in chronic myeloid leukaemia. Leukemia. 2016; 30: 1648-1671.
[135] Nakamura K, Johnson GL. PB1 domains of MEKK2 and MEKK3 interact with the MEK5 PB1 domain for activation of the ERK5 pathway. Journal of Biological Chemistry. 2003; 278: 36989-36992.
[136] Yang Q, Deng X, Lu B, Cameron M, Fearns C, Patricelli MP, *et al.* Pharmacological inhibition of BMK1 suppresses tumor growth through promyelocytic leukemia protein. Cancer Cell. 2010; 18: 396.
[137] Elkins JM, Wang J, Deng X, Pattison MJ, Arthur JSC, Erazo T, *et al.* X-ray crystal structure of ERK5 (MAPK7) in complex with a specific inhibitor. Journal of Medicinal Chemistry. 2013; 56: 4413-4421.
[138] Heo K, Cushman HJ, Akaike M, Woo C, Wang X, Qiu X, *et al.* ERK5 Activation in Macrophages Promotes efferocytosis and inhibits atherosclerosis. Circulation. 2014; 130: 180-191.
[139] Le N, Heo K, Takei Y, Lee H, Woo C, Chang E, *et al.* A Crucial role for p90RSK-mediated reduction of ERK5 transcriptional activity in endothelial dysfunction and atherosclerosis. Circulation. 2013; 127: 486-499.
[140] Kang C, Kim JS, Kim C, Kim E, Chung H. The pharmacological inhibition of ERK5 enhances apoptosis in acute myeloid leukemia cells. International Journal of Stem Cells. 2018; 11: 227-234.
[141] Shahidi F, Ambigaipalan P. Omega-3 polyunsaturated fatty acids and their health benefits. Annual Review of Food Science and Technology. 2018; 9: 345-381.
[142] Chang HY, Lee H, Kim W, Surh Y. Docosahexaenoic acid induces M2 macrophage polarization through peroxisome proliferator-activated receptor γ activation. Life Sciences. 2015; 120: 39-47.
[143] Alexander Aguilera A, Hernández Díaz G, Lara Barcelata M, Angulo Guerrero O, Oliart Ros RM. Induction of Cd36 expression elicited by fish oil PUFA in spontaneously hypertensive rats. the Journal of Nutritional Biochemistry. 2006; 17: 760-765.
[144] Madonna R, Salerni S, Schiavone D, Glatz J, Geng Y, Caterin R. Omega-3 fatty acids attenuate constitutive and insulin-induced CD36 expression through a suppression of PPARα/γ activity in microvascular endothelial cells. Thrombosis and Haemostasis. 2011; 106: 500-510.
[145] Franekova V, Angin Y, Hoebers NTH, Coumans WA, Simons PJ, Glatz JFC, *et al.* Marine omega-3 fatty acids prevent myocardial insulin resistance and metabolic remodeling as induced experimentally by high insulin exposure. American Journal of Physiology-Cell Physiology. 2015; 308: C297-C307.
[146] Adili R, Voigt EM, Bormann JL, Foss KN, Hurley LJ, Meyer ES, *et al. In vivo* modeling of docosahexaenoic acid and eicosapentaenoic acid-mediated inhibition of both platelet function and accumulation in arterial thrombi. Platelets. 2019; 30: 271-279.
[147] Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, *et al.* Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. New England Journal of Medicine. 2019; 380: 11-22.
[148] Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, *et al.* Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. The Lancet. 2007; 369: 1090-1098.
[149] Boden WE, Bhatt DL, Toth PP, Ray KK, Chapman MJ, Lüscher TF. Profound reductions in first and total cardiovascular events with icosapent ethyl in the REDUCE-IT trial: why these results usher in a new era in dyslipidaemia therapeutics. European Heart Journal. 2020; 41: 2304-2312.