The NO/cGMP/PKG pathway in platelets: The therapeutic potential of PDE5 inhibitors in platelet disorders

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Abstract
Platelets are the “guardians” of the blood circulatory system. At sites of vessel injury, they ensure hemostasis and promote immunity and vessel repair. However, their uncontrolled activation is one of the main drivers of thrombosis. To keep circulating platelets in a quiescent state, the endothelium releases platelet antagonists including nitric oxide (NO) that acts by stimulating the intracellular receptor guanylyl cyclase (GC). The latter produces the second messenger cyclic guanosine-3′,5′-monophosphate (cGMP) that inhibits platelet activation by stimulating protein kinase G, which phosphorylates hundreds of intracellular targets. Intracellular cGMP pools are tightly regulated by a fine balance between GC and phosphodiesterases (PDEs) that are responsible for the hydrolysis of cyclic nucleotides. Phosphodiesterase type 5 (PDE5) is a cGMP-specific PDE, broadly expressed in most tissues in humans and rodents. In clinical practice, PDE5 inhibitors (PDE5i) are used as first-line therapy for erectile dysfunction, pulmonary artery hypertension, and lower urinary tract symptoms. However, several studies have shown that PDE5i may ameliorate the outcome of various other conditions, like heart failure and stroke. Interestingly, NO donors and cGMP analogs increase the capacity of anti-platelet drugs targeting the purinergic receptor type Y, subtype 12 (P2Y12) receptor to block platelet aggregation, and preclinical studies have shown that PDE5i inhibits platelet functions. This review summarizes the molecular mechanisms underlying the effect of PDE5i on platelet activation and aggregation focusing on the therapeutic potential of PDE5i in platelet disorders, and the outcomes of a combined therapy with PDE5i and NO donors to inhibit platelet activation.

Keywords
Cyclic guanosine-3′,5′-monophosphate, nitric oxide donors, phosphodiesterase type 5, phosphodiesterase type 5 inhibitors, platelet disorders, thrombosis
INTRODUCTION

Platelets are small anucleated blood cells that ensure hemostasis at sites of blood vessel injury by aggregating and releasing a plethora of bioactive molecules, thereby limiting blood loss and pathogen entry, and promoting vessel repair.1

When the endothelial lining is intact and healthy, platelets are maintained in a resting non-adhesive state by short-lived molecules constitutively released by the endothelium, namely nitric oxide (NO) and prostacyclin (prostaglandin I2 [PGI2]). NO is an apolar gas produced from L-arginine by nitric oxide synthase (NOS) with an intravascular half-life of 2 ms that can enter platelets by simple diffusion and stimulate the intracellular soluble receptor guanylyl cyclase (sGC), which in turn catalyzes the conversion of guanosine-5′-triphosphate (GTP) to cyclic guanosine 3′,5′-monophosphate (cGMP).2 Prostacyclin is a lipid mediator of the eicosanoid family (half-life of 42 s) that binds the platelet G protein-coupled prostacyclin receptor (IP), which stimulates adenylyl cyclase to convert adenosine-5′-triphosphate (ATP) to adenosine 3′,5′-monophosphate (cAMP).3 cGMP and cAMP activate protein kinase G (PKG) and A (PKA), respectively, that phosphorylate hundreds of intracellular targets to ultimately activate the platelets flowing along the endothelium.4 The NO and PGI2 paracrine effect also extends to nearby leukocytes and smooth muscle cells.5 Thus, their overall outcome is to inhibit/dampen thrombosis and inflammation and promote vasodilation.

When the endothelial lining is injured or inflamed, the local concentration of these key inhibitory mediators is reduced locally and the molecular breaks are released and thereby smooth muscle cells contract and platelets and leukocytes are more prone to become active.6 At the sites of injury platelets sense and respond to exposed components of the extracellular matrix (ECM) such as collagen, and locally generated soluble agonists, such as thrombin; that is, the product of the coagulation cascade.7 These agonists trigger intracellular signaling cascades that promote the conversion of integrin receptors from a low- to a high-affinity state for their ligands (integrin inside-out activation).7 The β1 integrins (α2β1, α6β1, α5β1, αIIbβ3, αvβ3) support adhesion and spreading of the first layer of platelets to the ECM.8 αIIbβ3, the most abundant integrin expressed in platelets, in its active state binds to plasmatic fibrinogen or von Willebrand factor (VWF) released from the injured endothelium, supporting the formation of a three-dimensional platelet aggregate (hemostatic plug) at the injury site.9 The controlled amplification of activation that is necessary to recruit more platelets and to ensure stability of the growing aggregate is mediated by co-stimulatory signaling provided by the short-lived autocrine/paracrine agonists thromboxane (TxA2) and adenosine diphosphate (ADP), which are released from activated platelets and can only act locally like NO and prostacyclin.10,11

Uncontrolled activation of the platelet pro-adhesive and secretory functions is one of the main drivers of thrombosis and can also exacerbate other disease states such as inflammation, infections,12 diabetes, and cancer.13,14 For instance, COVID-19 patients display hyperactive platelets and a higher risk of thrombotic complications,15 which could be explained, at least in part, by the endothelial dysfunction and the reduced NO bioavailability documented in these patients.

In clinical practice, the strategy most widely used to counteract pathological platelet activation is to inhibit the amplificatory pathways mediated by TxA2 and ADP by treatment of patients with aspirin (which prevents TxA2 synthesis) and/or ADP receptor, purinergic receptor type Y, subtype 12 (P2Y12) blockers such as clopidogrel.15 In principle, one could lower platelet activation not only by inhibiting the activation pathways but also by boosting the inhibitory pathways.

One way to do this is by increasing the intracellular levels of cGMP. Intracellular cGMP pools are tightly regulated by a fine balance between the NO receptor guanylyl cyclase (GC) and phosphodiesterases (PDE) that are responsible for cyclic nucleotides hydrolysis.16 In mammals, the PDE superfamily includes 60 different isoforms with different specificity for cAMP and/or cGMP.17 Phosphodiesterase type 5 (PDE5) is a cGMP-specific phosphodiesterase broadly expressed in most tissues in humans and mice.18–20 which jumped to the headlines thanks to the development of its pharmacological inhibitor, sildenafil (Viagra). In clinical practice PDE5 inhibitors (PDE5i) are used as first-line therapy for erectile dysfunction (ED), pulmonary arterial hypertension (PAH), and lower urinary tract symptoms (LUTS).21 However, several studies have shown that PDE5 may ameliorate the outcome in various other conditions, including heart failure,22 stroke,23 diabetic nephropathy,24,25 peripheral artery disease, and premature ejaculation,26 and its clinical applications are increasing in several fields.27–30

As expected, NO donors and cGMP analogs greatly increase the capacity of P2Y12 inhibitors to block platelet aggregation31 and PDE5i in particular have been shown to inhibit platelet function.32 However, the pathological context in which these therapeutic strategies could be most effective and safe remains to be established.

SIGNaling PATHWAYS REGULATING CGMP in PLatelets

2.1 | Nitric oxide

Cytosolic levels of cGMP are regulated by a fine balance between the activity of the NO-stimulated sGC and PDEs. The harmonized activity of these players converges in a complex signaling pathway whose initiator is NO, a free radical, naturally produced through oxidation of the amino acid L-arginine by the NOS. The main source of NO in the blood is endothelial NOS (eNOS), but there is also a NOS resident in platelets that contributes to the vascular pool of NO.33 NO regulation of vascular tone occurs through different pathways according to cell needs and environmental stimuli. Under physiological conditions, NO mainly synthesized by endothelial eNOS induces cGMP production via stimulation of sGC in vascular smooth muscle cells.34 The increase of cGMP activates PKG thus...
preventing calcium influx and promoting cytosolic calcium reuptake into the sarcoplasmic reticulum. These coupled events, acting on myosin-actin bridges, finally induce muscle relaxation. Under hypoxic conditions sGC-mediated cGMP production is inhibited and Rho-associated protein kinase (ROCK) activation mediated by inosine cyclic monophosphate (cIMP) triggers muscle contraction.\textsuperscript{35} These mechanisms ensure vascular homeostasis and prevent vascular damage that occurs in several pathological conditions such as atherosclerosis in which NO concentration declines promoting smooth muscle cell proliferation and ECM deposition.\textsuperscript{5} NO is best known for its stimulatory role on sGC. However, NO can downregulate platelet function, independently of sGC, through nitration or S-nytrosilation of intracellular signaling proteins.\textsuperscript{36,37} S-nytrosilation of N-ethylmaleimide–sensitive factor (NSF) was shown to inhibit platelet granule release, indispensable for platelet activation, by impairing its ability to disassemble the soluble N-ethylmaleimide NSF attachment receptor (SNARE) complex.\textsuperscript{36} Even though it is well established that NO is a strong negative regulator of platelet activation and adhesion,\textsuperscript{38,39} under oxidative stress conditions, there is an impairment of NO signaling in platelets called “NO resistance” leading to dysfunctional classical NO/cGMP/PKG pathway. Superoxide anion ($O_2^-$) radicals can act as NO scavengers and at the same time inactivate sGC.\textsuperscript{40}

Platelet sensitivity to NO as well as cGMP and sGC decreases with age, and among young people, women are the ones to show a higher expression of cGMP and sGC, making it a more reliable role of the NO/GC/cGMP axis in platelets.\textsuperscript{47,49} Consistently, NO-independent pharmacological sGC agonists inhibit platelets.\textsuperscript{50} They can be distinguished in sGC stimulators and sGC activators that are effective on Fe$^{2+}$ or Fe$^{3+}$ iron, respectively. A known sGC stimulator, YC-1 (3-[5-hydroxymethyl-2furyl]-1-benzyl indazole), acts on sGC by sensitizing it to CO, stimulating sGC in a NO-independent manner.\textsuperscript{51} sGC stimulators also increase sGC activity acting on specific regions in the structure of the enzyme, such as BAY 41-2272, which acts on the regulatory region on the sGC $\alpha_1$ subunit, increasing the enzyme activity.\textsuperscript{52} Activation of the sGC can be induced in a heme-dependent or -independent manner. The heme-dependent activation of sGC is observed during NO binding to Fe$^{2+}$,\textsuperscript{13} or during treatment with nitrovasodilators such as glyceryl trinitrate, that result in activation of sGC by modulating the heme moiety, forming a nitrosyl heme of the enzyme.\textsuperscript{54} YC-1, on the other hand, activates sGC in both NO-dependent and NO-independent manners.\textsuperscript{55} The sGC activator HMR1766 reduces platelet activation in vivo after chronic treatment of diabetic rats.\textsuperscript{56} The sGC activator BAY 60-2770 was shown to overstimulate platelet sGC and to inhibit platelet aggregation, adhesion, intracellular Ca$^{2+}$ levels, and integrin $\alpha$IIb$\beta$3 activation particularly in heme-oxidizing conditions.\textsuperscript{57} The GC stimulator riociguat is effective in the treatment of pulmonary hypertension but it was shown to inhibit platelet activation in whole blood only at concentrations above 50$\mu $M, which is much higher than the concentrations reached in the plasma of patients (150–500 nM), suggesting that platelets may not be the target of this drug.\textsuperscript{58} On the other hand, the phosphodiesterase 3 (PDE3) inhibitor dipyridamole, a widely used antiplatelet drug, has been shown to augment NO production and bioavailability under diabetic conditions\textsuperscript{59} and on experimental models of ischemic limbs.\textsuperscript{60} In comparison to riociguat, dipyridamole is more effective because it increases NO bioavailability; inhibits the cellular reuptake of adenosine, thus inhibiting platelet aggregation; and has been shown to play an effective role in platelets, where it inhibited shear-induced platelet aggregation with a larger efficacy in whole blood compared to platelet rich plasma.\textsuperscript{61} making it a more reliable treatment for platelet-related disorders. Thrombospondin-1 (TSP-1) is a universal inhibitor of sGC that blocks both heme-dependent and -independent activation.\textsuperscript{62} TSP-1 increases with age, and in chronic diseases such as diabetes and atherosclerosis, thus the therapeutic potential of drugs that target sGC could be compromised in these pathological conditions in which TSP-1 signaling is elevated.\textsuperscript{62} While the inhibitory effect of sGC in NO-stimulated circulating platelets is unquestionable, its role during thrombus formation seems to be more complex. Increasing evidence suggests that sGC can be activated downstream of the VWF receptor GP-Ib-IX-V$\alpha_2$ or the Toll-like receptor
(TLR)4 stimulated by pathogen-associated molecular patterns (PAMPs) like lipopolysaccharide or damage-associated molecular patterns (DAMPS) and can have a stimulatory effect on platelet activation in synergy with other platelet agonists. Moreover, a recent study has shown that sGC has mechanosensitive properties and its inhibitory function is potentiated by shear stress. These apparently contradictory observations are conciliated by a proposed model in which, during thrombus formation, sGC might display a biphasic role in the modulation of platelet activation. In the early stages of thrombus formation, platelet sGC/cGMP axis is activated by VWF or PAMPs and DAMPs situated at the site of injury and promotes thrombus formation. During the late stages of thrombus growth, the effect of high shear stress stimulates the sGC/cGMP inhibitory signaling axis and results in the limitation of platelet activation and of thrombus growth. NO-dependent or -independent activation of sGC leads to increase of cGMP, whose levels are finely regulated by the action of cGMP-hydrolyzing phosphodiesterases.

2.3 | Phosphodiesterases and platelets

Phosphodiesterases belong to a large family of enzymes virtually expressed in all tissues and responsible for cyclic nucleotide hydrolysis. Eleven PDE families have been identified so far that, following multiple splicing processes, give rise to more than 100 PDE isoforms in humans and rodents. PDEs differ in their structure, properties, location, cellular expression, and targets. PDE5 is the principal cGMP hydrolizer, for which three isoforms have been identified in humans and mice. Given the broad expression and the ability of PDE5 to control cGMP levels in the cell, it has been proposed as a pivotal effector in many biological processes such as platelet activation and aggregation, smooth muscle relaxation, immune response, and heart muscle contraction.

Three PDE isoenzymes are expressed in platelets, PDE2, PDE3, and PDE5, and while PDE2 hydrolyzes cAMP, and PDE3 both cAMP and cGMP, PDE5 is a cGMP-specific enzyme. The high expression of PDE5 in platelets leads to rapid cGMP hydrolysis, allowing platelets to promptly activate and aggregate when the endothelium is injured.

2.4 | cGMP effectors

The basal cGMP level in platelets is reported to be around 0.5 μM. NO-mediated activation of sGC results in a rapid 10-fold increase in cGMP levels. This transient elevation is mirrored by activation and subsequent phosphorylation of PDE5. Phosphorylation of PDE5 has been suggested to enhance cGMP hydrolysis in vitro. Since its discovery in rat urine about six decades ago, various studies have been conducted to better understand cGMP’s effect on platelet function. cGMP intracellular levels in platelets are tightly controlled by the activity of sGC and cGMP-hydrolyzing PDEs.

The main effector of cGMP is PKG that inhibits platelet activation by phosphorylating multiple substrates. One of its targets is IP3R-associated PKG substrate (IRAG) that results in the inhibition of IP3-dependent Ca2+ release from the intracellular stores. By blocking Ca2+ mobilization, cGMP negatively regulates all aspects of platelet activation, from integrin activation to cytoskeleton remodeling and granule release. In addition, phosphorylation of regulatory proteins of Ras-like guanine-nucleotide-binding protein (Rap1GAP2) and of RhoA (Myo9b and GEF-H1) contributes to the inhibition of platelet aggregation and of cytoskeletal dynamics. Additionally, PKG was shown to inhibit the platelet procoagulant response (PS exposure, mitochondrial membrane depolarization) but its impact on platelet lifespan is yet to be determined. A cross-talk between cGMP and cAMP has been reported in many tissue and cell types including platelets. Most notably, cGMP inhibits PDE3, which degrades cAMP, and stimulates PDE2, which degrades both cGMP and cAMP, with the final effect of increasing cAMP. Thus, cGMP inhibits platelet activation in a PKG-dependent (NO/cGMP/PKG) and PKG-independent (NO/cGMP/PDE3A/cAMP/PKA) manner (Figure 1). Moreover, PKA is able to phosphorylate and activate PDE5 on the PKG site (Ser92), but with 10-fold lower efficiency than PKG. Only a few substrates are known to be selectively phosphorylated by PKA (i.e., PDE3, IP3R, TRPC, MLCK) and PKG (i.e., PDE5 and IRAG), while PKA and PKG can phosphorylate both the same substrates including the vasodilator-stimulated phosphoprotein (VASP), LIM and SH3 domain protein (LASP), and heat shock protein 27 (HSP27). The NO/sGC/cGMP pathway plays an important role in the activity of different cell types, organs, and tissues, most importantly in the brain, the corpus cavernosum, and the cardiovascular system. In platelets, studies in humans and genetically modified animal models have demonstrated that upregulating the cGMP/PGK pathway is critical for reducing platelet aggregation and the risk of myocardial infarction.

3 | The Therapeutic Potential of PDE5 Inhibitors in Platelet Disorders

PDE5 inhibitors reached their breakthrough at the end of the last century and are currently used as first-line therapy in patients with ED, PAH, and LUTS. The most widely used PDE5i, sildenafil, commonly known as Viagra, was discovered in 1989. Its ameliorating outcomes in patients with ED depend on its vasodilating properties, which are beneficial also in other clinical settings including cardiovascular disease (CVD). In preclinical studies, PDE5 inhibitors’ effects on thrombosis have been examined in an experimental rat model of thrombotic suture suggesting a benefit in applying sildenafil in the anastomosis with already present thrombogenic disease. The same effect is shown in clinical studies in which sildenafil reduced thrombosis, thromboembolic events, and the risk of thrombotic strokes in patients during low-level hemolysis (LLH) on HeartMate II support. Moreover, a retrospective analysis of clinical studies assessing the potential effect of PDE5i
in ED suggests that use of sildenafil improves the outcome of CVD patients\textsuperscript{96} and that there should be no safety concerns in the use of this drug in CVD patients.\textsuperscript{97}

An analysis of a Dutch registry (INTERMACS) of patients that used PDE5i after left ventricular assist device placement demonstrates that PDE5 inhibition improves survival and reduces thrombotic events.\textsuperscript{98} Thus, the reduction in CVD risk is not only due to the vasodilating effect of PDE5i but also to their ability to inhibit platelet function.\textsuperscript{99} Even though sildenafil shows several beneficial effects, its short half-life\textsuperscript{100} has led to the development of other PDE5 inhibitors, such as tadalafil, vardenafil, and avanafil with longer half-lives.\textsuperscript{21} Tadalafil has shown promising results in ex vivo studies and clinical trials, where its administration inhibits platelet activation, increases cGMP levels,\textsuperscript{101} and reduces inflammation after treatment with 5 mg/day tadalafil.\textsuperscript{102}

Vardenafil is not only more effective than sildenafil in extending NO-induced relaxation of trabecular smooth muscle cells, but can also inhibit Ca\textsuperscript{2+} flux, thereby inhibiting platelet activation.\textsuperscript{103}

\section*{FIGURE 1}\textsuperscript{107} Nitric oxide (NO)/cyclic guanosine monophosphate (cGMP)/protein kinase G (PKG) transduction signaling pathway in platelets. NO is produced by oxidation of L-arginine by the NO synthase (NOS) enzymes, and acts as an activator for soluble guanylyl cyclase (sGC), which induces cGMP production through guanosine-5'-triphosphate (GTP) phosphorylation. Many NO donors, endogenous and exogenous ones, augment NO bioavailability, thus leading to a major activation of the cGMP pathway. cGMP can both inhibit and enhance cyclic adenosine monophosphate (cAMP) production in platelets, by allosterically inhibiting phosphodiesterase 3 (PDE3)—a cAMP inhibitor, and by stimulating phosphodiesterase 2 (PDE2) that degrades cAMP, so NO/cGMP can inhibit platelet activation either in a cGMP-dependent PKG-dependent (NO-cGMP-PKG) or -independent (NO-cGMP-PDE3A-cAMP-PKA) pathway. cGMP binds to the three different effector proteins, PKGs, PDEs, and the cyclic nucleotide-gated cation channels (CNG channels) that mediate sensory transduction in cells.

Phosphodiesterase 5 (PDE5) is a cGMP-specific phosphodiesterase that targets it and inhibits the crucial NO/cGMP/PKG signaling pathway. In presence of PDE5 inhibitors (PDE5i), PDE5 cannot exert its hydrolyzing function, by allowing cGMP to continue its platelet inhibitory function through PKG. AMP, adenosine monophosphate; ATP, adenosine triphosphate; PS, lipopolysaccharide; PKA, protein kinase A.

\section*{4 | PDE5 INHIBITORS COMBINED WITH NO DONORS COULD HAVE A SYNERGIC EFFECT ON PLATELET INHIBITION}

PDE5 inhibitors alone have displayed limited effect as platelet aggregation inhibitors.\textsuperscript{22} It has been demonstrated that in platelets, the presence of NO is able to induce an increase of cGMP content promoting the intracellular accumulation of PDE5i raising their affinity for PDE.\textsuperscript{104} Moreover, a synergic effect of PDE5i in combination with NO for the treatment of COVID-19 has been proposed\textsuperscript{28} and sildenafil has been shown to prompt the anti-aggregatory effect of NO donors that inhibit platelet activation and aggregation through cGMP-dependent and -independent pathways.\textsuperscript{105,106}

A more recent study\textsuperscript{107} has shown very promising outcomes on ED patients by combining low-dose tadalafil with dietary nutritional supplements that can boost the endothelial NO production, such as Panax ginseng, traditionally used for its properties as a vascular endothelial cell-derived NO secretion promoter; moringa oleifera,
known for its anticoagulant properties; and rutin, which possesses anticoagulant and anti thrombotic properties. Another study has shown that the use of compounds that augment NO levels and eNOS activity, such as endocannabinoid anandamide, shows an effect on platelet NO/cGMP pathway, by increasing in a dose- and time-dependent manner NO and cGMP levels in human platelets and stimulating eNOS activity, contributing to an extended platelet survival.

All together, these data suggest a potential beneficial effect of PDE5 inhibitors combined with NO donors in platelets. However, dedicated studies deeply assessing this concern are missing.

5 | WHO SHOULD TAKE PDE5 INHIBITORS AS ANTI-PLATELET THERAPY?

The inhibitory effect of PDE5i on platelet aggregation suggests that these drugs alone or in combination with other therapies could be employed in the setting of CVD to reduce the risk of thrombosis and minimize the infarct size. However, randomized clinical trials examining the safety and efficacy of PDE5i in CVD are still lacking.

The TARDIS trial showed that combining aspirin, clopidogrel, and dipyridamole, a pan-PDE inhibitor, did not reduce the incidence and severity of recurrent stroke compared to clopidogrel alone or aspirin and dipyridamole combined, but significantly increased the chance of bleeding. Thus, further trials are needed to find the clinical setting, modalities, and timing of administration in which these drugs could be most beneficial. Another recent study shows how the differential reactivity of platelets in males and females is at least in part due to differences in the NO–sGC signaling pathway, and that women are indeed prone to benefit from larger doses of antiplatelet drugs that block the P2Y12 receptor.

The L-arginine/NO/cGMP pathway is impaired in platelets from obese adults, associated with reduced superoxide dismutase (SOD) activity, resulting in reduced NO bioavailability that supports platelet hyperaggregation. Thus, among obese patients, combination therapy with NO donors and PDE5 could be preferable. In addition to their ability to form thrombi, platelets can also foster pathological conditions through their capacity to sense the activated endothelium, release chemokines, and bind leukocytes. In the setting of the arteries, platelets participate in the initial stages of atherosclerosis by arresting leukocytes and facilitating their transmigration at sites of endothelial activation. In veins where the endothelium is activated by flow stagnation and hypoxia platelets activate neutrophils to release neutrophil extracellular traps, which in turn promote thrombosis. After stroke, platelets contribute to ischemia reperfusion injury and stroke progression through the recruitment of T cells. The common feature of these pathological states is endothelial dysfunction that results in reduced bioavailability of the platelet antagonists NO and PGI2 and decrease of the platelet activation threshold.

Notably, a recent study demonstrated that tadalafil does not affect platelet adhesion to collagen and thrombus formation (platelet functions required for hemostasis) but significantly reduces platelet adhesion to inflamed endothelial cells and release of chemokines from activated platelets (platelet functions that foster inflammation). Because tadalafil also reduces inflammation, one could envision the use of this drug to prevent the progression of thrombo-inflammatory disease without undermining hemostasis. However, these studies need confirmation among human subjects.

6 | CONCLUSIONS

This review dissects the NO/cGMP/PKG/PDE5 pathway and its relevance in platelet physiopathology, and summarizes recent findings coming from preclinical studies that might shed light on the therapeutic potential of PDE5i as anti-platelet treatment. From the analysis of available studies, the increase of cGMP levels through PDE5i or sGC stimulation emerge as a promising therapeutic tool in platelet disorders. However, randomized controlled trials are needed to assess efficacy and safety of these treatments.

AUTHOR CONTRIBUTIONS
AD and FC did the literature research and wrote the first draft of the review. AD and FC designed the figure. LS and MAV performed critical revision for important intellectual content. All the authors reviewed and approved the final version of the manuscript for consideration of publication.

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