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Methods for measurement of platelet function in the assessment of nonclinical drug safety and implications for translatability

David Ledieu and Serge Côté

Abstract

Platelets play a pivotal role in normal hemostasis. Drug-induced derangement of platelet function can lead to either an increased bleeding risk when platelet function is inhibited or a proaggregant state that can manifest as thrombosis when it is exacerbated. In both cases, drug-induced platelet dysfunction can lead to serious adverse events in patients that can limit drug prescription or ultimately lead to the withdrawal of the drug from the market. Despite those risks, drug-induced platelet function defects do not appear to be highlighted during drug development; rather they are reported at the postapproval stage indicating that current preclinical assays and clinical development studies are failing to capture these liabilities. However, significant progresses have been made in platelet function testing and clinically useful methods now exist for the measurement of platelet function. This review article discusses these methods and describes their advantages and disadvantages in the setting of nonclinical drug safety to assess drug-induced platelet dysfunction and on the translatability of these tests to predict thrombosis and bleeding in patients.

Addresses

Translational Medicine, Novartis Institutes of Biomedical Research, Basel, Switzerland

Corresponding author: Ledieu, David (david.ledieu@novartis.com)

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Platelet function, Bleeding, Thrombosis, Platelet function assays, Nonclinical drug safety.

Introduction

Platelets play a pivotal role in normal hemostasis. They are required for the formation of primary hemostatic plugs to repair small vascular defects; they provide a surface for coagulation, amplifying minute stimuli into explosive production of fibrin to form more stable secondary hemostatic plugs; finally, they are involved in clot retraction and in the release of promoters of endothelial repair, facilitating wound closure, and restoration of normal vessel architecture [1–3]. In addition, platelets play a central role in the pathogenesis of thrombotic diseases and associated clinical ischemic events [4]. Besides these long-established roles in hemostasis and thrombosis, platelets are increasingly recognized as pivotal players in numerous other pathophysiological processes including inflammation and atherogenesis, antimicrobial host defense, and tumor growth and metastasis [5]. Because of their dynamic and multifunctional nature, and the redundancy of their molecular signaling pathways with other cell types and, for instance, inflammatory cells that can be targeted by some drugs, many drugs can interact with platelets and affect potentially multiple aspects of their hemostasis function. A list of the drugs with a demonstrated or suspected effect on platelet function is provided in Table 1. Some drugs may be deliberately designed to alter and correct platelet function as for antiplatelet therapy. In that case, as platelet function inhibition is the expected pharmacological effect, it is thoroughly evaluated during the development of these drugs as a key efficacy readout. The bleeding risk associated with antiplatelet therapy is well known and it is anticipated in patients who are closely monitored to avoid any adverse events. In contrast, some drugs can unexpectedly inhibit or stimulate platelet function leading to an increased risk of either bleeding or thrombosis [6]. In most of the cases, this undesired effect is not anticipated and therefore it is not specifically evaluated during drug development, neither in clinical trials nor in preclinical toxicity studies. If severe enough, a drug-induced platelet dysfunction may induce adverse events such as hemorrhages or thrombosis in preclinical toxicity studies, or in healthy volunteers, or ultimately in the limited number of patients treated during the clinical trials. Otherwise, this safety concern might be discovered only when the drug will reach the market with a sufficiently high number of patients treated and with some of them bearing additional risk factors that will potentialize the risk of bleeding or thrombosis (e.g., concurrent antiplatelet or anticoagulant therapy, congenital hemostasis disorders, hypercoagulable state associated

Table 1 List of selected drugs that induce platelet dysfunction with possible related safety concern in patients.

Drug class	Specific agents	Mechanism of action	Effect in patients	Selected references
Examples of drugs that induce platelet aggregation				
	Heparin	Platelet activation by direct binding of heparin; Activation of platelet surface FcγRIIa by heparin ICs	Nonimmune heparin-associated thrombocytopenia; Heparin-induced thrombocytopenia type II; Thromboembolism	[10,11]
Therapeutic antibodies	hu5c8 anti-CD40L antibody	Activation of platelet surface FcγRIIa by anti-CD40L ICs	Thromboembolism	[12–14]
	Avastin	Activation of platelet surface FcγRIIa by bevacizumab ICs	Increased thrombosis risk[b]	[15]
	Intravenous immunoglobulin	Activation of platelet surface FcγRIIa by aggregated IgGs	Thromboembolism[b]	[16,17]
Nanoparticle-based drugs		Platelet activation and aggregation[a]		[18]
Examples of drugs that enhance platelet aggregability				
	Cyclosporin A	Increased platelet aggregation[a]	Increased thrombosis risk[b]	[19,20]
	Tegaserod/5-HT4 agonist	Increased platelet aggregation	Increased thrombosis risk[b]	[21]
	Cisplatin	Increased platelet activation[a]	Increased thrombosis risk[b]	[22]
Examples of drugs that inhibit platelet aggregability				
Antiplatelet drugs	Aspirin/acetylsalicylic acid, triflusal	Irreversible cyclooxygenase inhibition	Increased bleeding risk	[23]
	Cangrelor, clopidogrel, prasugrel, ticagrelor, ticlopidine	Adenosine diphosphate receptor inhibition	Increased bleeding risk	
	Cilostazol	Phosphodiesterase inhibition	Increased bleeding risk	
	Vorapaxar	Protease-activated receptor-1 antagonism	Increased bleeding risk	
	Abciximab, eptifibatide, tirofiban	Glycoprotein IIb/IIIa inhibition	Increased bleeding risk	
	Dipyridamole	Adenosine reuptake inhibition	Increased bleeding risk	
	Terutroban	Thromboxane inhibition	Increased bleeding risk	[24]
Nonaspirin nonselective nonsteroidal anti-inflammatory drugs	Piroxicam, ibuprofen, naproxen, tiaprofenic acid, indomethacin, etc.	Reversible cyclooxygenase inhibition	Increased bleeding risk	[25,26]
Tyrosine kinase inhibitors	BTKi/ibrutinib or BCR-ABLi/imatinib, dasatinib	Platelet protein kinase inhibition	Increased bleeding risk	[27,28]
Serotonin reuptake inhibitors	Escitalopram, sertraline, citalopram, fluoxetine, paroxetine, venlafaxine, duloxetine, sibutramine	Inhibition of serotonin reuptake into platelets	Increased bleeding risk	[29–31]
Calcium channel blockers	Verapamil, nifedipine, diltiazem	Platelet calcium influx inhibition[a]	Increased bleeding risk	[32,33]
Antibiotics	Beta-lactam antibiotics	Thromboxane inhibition, platelet calcium influx inhibition[a]	Increased bleeding risk	[34–36]
	Cephalosporins	Platelet agonist receptor inhibition[a]	Increased bleeding risk[b]	[37]
Anesthetics	Halothane, propofol	Platelet aggregation inhibition[a]	Increased bleeding risk[b]	[38,39]
Chemotherapeutic drugs	1,3-Bis(2-chloroethyl)-1-nitrosurea, cyclophosphamide	Platelet aggregation inhibition[a]	Increased bleeding risk[b]	[40]
	Daunorubicin	Platelet aggregation inhibition[a]	Increased bleeding risk[b]	[41]

ICs, immune complexes; IgG, immunoglobulin G.

[a] Indicates that the precise mechanism contributing to platelet dysfunction is not known.

[b] Indicates that the relevance of the platelet dysfunction to patient safety is uncertain.

with cancer or chronic inflammatory diseases, etc.). This is obviously too late and an important concern for patient safety. Thus, although drug-induced platelet dysfunction and related hemostasis impairment are a very pertinent safety concern, these are currently poorly and inconsistently evaluated in drug safety, and particularly in nonclinical drug safety where hemostasis testing is often limited to the standard coagulation tests including prothrombin time, activated partial thromboplastin time, and thrombin time expressed as clottable fibrinogen concentration, and platelet count, whereas platelet function is not assessed routinely [7–9].

Meanwhile, progresses have been made in platelet function testing and clinically useful methods now exist for the measurement of platelet function that can be also used in laboratory animal species. This review article discusses these methods and describes their advantages and disadvantages in the setting of nonclinical drug safety to assess drug-induced platelet dysfunction and on the translatability of these tests to predict thrombosis and bleeding in patients. In addition, a number of bleeding or thrombosis animal models have been developed providing useful tools to evaluate drug-induced platelet dysfunction and their possible consequences on hemostasis. However, those in vivo models were considered beyond the scope of this review.

General considerations on platelet function testing in nonclinical drug safety assessment

Overall, performing platelet function assays in laboratory animals is similar to in humans. However, methods need to be adapted to accommodate their different platelet concentration and size, and some interspecies differences in platelet biology and receptor pathways reflecting either structural differences (including composition of the platelet membrane and presence of specific agonist receptors), or activation of distinct signaling pathways by the agonist [42–44].

The volumes of blood required for most of platelet function tests and their relative low throughput were also important barriers to the utilization of platelet function assays in laboratory animals (and especially in rodents) and in the setting of preclinical toxicity studies. The new generation of standard platelet function analyzers (PFAs) as well as new analytical methods (e.g., flow cytometry [FC]- or plate-based assays) are now much better adapted to the use in laboratory animals.

Platelet function tests reported in this review are listed by their different method principles and applications, and the pros and cons of their use in nonclinical drug safety assessment in Table 2.

Standard platelet function testing assays

Bleeding time tests

With the bleeding time (BT), a standardized and uniform cut on the skin, oral mucosa, tail, or nail is made, and the time taken to stop bleeding is measured. BT has the advantage to be easy and quick to perform without any blood sample processing. It requires, however, a well-standardized test procedure with an accurate timing. Cutaneous and mucosa BT tests are used in large animals and mainly assess primary hemostasis integrity involving capillaries and microvessels of the superficial area of the skin or mucosa but not veins or arteries. In that situation, primary hemostasis with the formation of the primary platelet clot is sufficient enough to stop hemorrhage without the need for activation of coagulation. This is not the case anymore with the tail or nail BT tests which are usually used in small animal species and involve larger vessels with the need of stabilization of the primary platelet clot with the formation of insoluble, cross-linked fibrin by activation of coagulation factors, specifically thrombin [45,46]. Automated incision-making devices for human use (e.g., Surgicutt®) can be used in large animals to standardize the test [47]. When the test is done on anesthetized animals (as usually in rodents), possible interference of anesthetic agents on platelet function may occur. Owing to lack of accuracy, sensitivity, and unclear predictive value for the risk of bleeding in patients, the BT test is not used routinely in clinical practice to assess platelet function [48]. However, this is precisely because of its lack of sensitivity that it should be regarded in preclinical studies as a good screening test to assess drug-induced platelet inhibition and related bleeding risk. A positive test with a clear treatment-related increase in BTwill suggest a severe defect of platelet function. The tail BT in rodents assessing not only primary hemostasis but also coagulation will be even less sensitive to detect a defect in platelet function. However, it provides a good way to assess the global effect of a compound on the whole hemostasis process with certainly a high positive predictive value for the risk of bleeding [43,49,50].

Flow cytometry–based assays

If FC is a powerful analytical tool to evaluate immune responses in preclinical drug development [51,52], it is increasingly being used for other purposes including platelet function evaluation. FC can monitor a whole panel of platelet activation markers and platelet interactions with other cells. This analysis is based on the optical and fluorescence evaluation of physical (size and internal complexity) and antigenic properties of platelets. It can quantitate receptor expression and inhibition, conformational changes related to platelet activation, platelet granule secretion, platelet microparticles, platelet aggregates, leukocyte–platelet aggregates, and even platelet reactivity (in agonist-stimulated whole blood [WB] samples) [53]. Most of those assays have been adapted to the use in laboratory

Table 2 List of tests for measurement of platelet function and their pros and cons in the assessment of nonclinical drug safety.

Test	Test sample	Platelet function tested	Test principle	Pros	Cons
Bleeding time tests					
Cutaneous and mucosa bleeding times	Native WB	Primary platelet clot formation	In vivo measurement of bleeding cessation	In vivo tests Quick and easy No sample processing High positive predictive value	Poorly standardized High analytical variability Lack of sensitivity (Low negative predictive value)
Tail and nail bleeding times	Native WB	Primary platelet clot formation and coagulation	In vivo measurement of bleeding cessation	In vivo tests No sample processing Global hemostasis test High positive predictive value	Invasive Poorly standardized High analytical variability Lack of sensitivity (low negative predictive value)
Tests based on platelet aggregation					
Light transmission aggregometry	Citrated PRP, washed platelets	Platelet aggregation	Photo-optical measurement of light transmission in relation to agonist-induced platelet aggregation	Historical gold standard Clinical diagnostic method Platelet activation pathways investigated independently	Time-consuming Large sample volume Preanalytical variability (Sample collection and processing) Sample stability High analytical variability Interspecies variability Specialized technical staff
Impedance whole blood aggregometry (WBA)	Citrated, hirudinized, or heparinized WB	Platelet aggregation	Measurement of electrical impedance in relation to agonist-induced platelet aggregation	WB test No sample processing Clinical diagnostic method Platelet activation pathways investigated independently	Large sample volume Fresh WB sample required (Sample stability) Preanalytical variability (Sample collection) High analytical variability Interspecies variability Specialized technical staff
Tests based on flow cytometry	Citrated WB, Citrated PRP, Washed platelets	Cell counting, platelet activation detection by extent of expression of surface and/or cytoplasmic biomarkers	Laser-based detection of fluorescent labeled platelet suspension in a flowing solution	Widely available platform WB tests Small sample volume Flexible	Time-consuming Preanalytical variability (sample collection) Sample stability Specialized technical staff
Tests based on platelet adhesion under shear stress					
PFA-100®	Citrated WB	Platelet adhesion and aggregation	Time evaluation of platelet plug formation to block a hole in activated surface under high-shear condition	WB test In vitro standardized BT Quick and easy High shear stress Clinical diagnostic method	Large sample volume Fresh WB sample required (sample stability) Low throughput (point-of-care system) Rigid closed system

Tests based on viscoeslastometry					
Thromboelastography (TEG®)	Citrated or heparinized WB	Platelet function contribution to clot formation, clot retraction, and clot lysis	Evaluation of rate and quality of clot formation under low shear condition	Global hemostasis test High positive predictive value	Large sample volume Fresh WB sample required (Sample stability) Low throughput (Point-of-care system) Lack of sensitivity to detect impaired platelet function (Low negative predictive value)
Thromboelastometry (ROTEM®)	Citrated or heparinized WB	Platelet function contribution to clot formation, clot retraction, and clot lysis	Evaluation of rate and quality of clot formation under low shear condition	Global hemostasis test High positive predictive value Automated pipetting and ready-to-use reagents	Fresh WB sample required (sample stability) Lack of sensitivity to detect impaired platelet function (Low negative predictive value)

Abbreviations: BT, bleeding time; PRP, platelet-rich plasma; WB, whole blood.

animals [54—58]. However, the most common use of FC in the setting of nonclinical drug safety is to assess the level of platelet activation in WB using the internal alpha-granule membrane protein CD62P (P-selectin) platelet surface expression. The lysosomal integral membrane protein CD63 platelet surface expression or the activation of the glycoprotein IIb/IIIa complex (measured by its specific binding to the monoclonal antibody PAC-1) can also be used but less commonly. FC has many advantages, such as the use of small volumes of blood and the analysis of platelets in whole blood, and FC capabilities are commonly available in most of preclinical safety departments. On the other hand, FC analysis is a relatively expensive and time-consuming test, requiring specialized operators.

Platelet aggregation tests

Light transmission aggregometry (LTA) is the historical gold standard test for platelet function evaluation [59]. The assay is based on the measurement of the increase in light transmission through the optically dense sample of platelet-rich plasma (PRP) or washed platelets after the addition of an exogenous platelet agonist and the precipitation of platelet aggregates. By using a large panel of agonists (e.g., adenosine-diphosphate [ADP], arachidonic acid, collagen, epinephrine, thrombin receptor activating peptides, ristocetin), information can be obtained about the various platelet activation pathways. Although LTA is regarded as a gold standard test, the assay may be affected by different preanalytical and analytical conditions (i.e., type of anticoagulant, hemolytic or lipemic plasma, PRP preparation, agonist concentrations), and the laboratory staff should be highly specialized in performing and interpreting the data. LTA has been adapted to a 96-well plate format (optical multichannel [Optimul] platelet aggregometry), providing a high throughput standardized method with a very small sample volume requirement [60]. Recently, the Optimul method has been adapted to measure aggregation in mouse platelets, offering a viable, standardized approach, allowing platelet function testing without the need for dedicated equipment [44].

With impedance whole blood aggregometry (WBA), platelet function is evaluated in saline-diluted WB. In this case, the assay is based on the measurement of the increase in electrical impedance between two electrodes as activated platelets stick to their artificial surface, and aggregate and accumulate around the electrodes after agonist induction [61]. By using WB, platelet function is assessed under more physiological conditions, given that the other blood elements may also affect platelet function. In addition, with the use of WB versus PRP samples, sample processing is limited avoiding the loss of larger platelets and platelet activation during PRP preparation. The multiple electrode aggregometry is a new generation of WBA with a

new pertinent device (Multiplate®, Roche Diagnostics International Ltd, Rotkreuz, Switzerland). It is a five-channel computerized instrument equipped with disposable cuvettes containing two independent electrode pairs serving as internal control and a stirring bar, as well as ready-to-use reagents (diluents and agonists), and automated pipetting that helps to reduce analytical variation [62]. Both LTA and WBA methods have been established in multiple laboratory animal species offering a translational approach to assess platelet aggregation in preclinical studies [42,63–65].

Tests based on platelet adhesion under shear stress

The PFA-100® or the newly available and upgraded Innovance PFA-200®, (Siemens, Munich, Germany) has been regarded as the standardization of BT, offering higher sensitivity [66]. The method is based on the property of platelets to adhere upon shear stress conditions and aggregate in the system in the presence of agonist. The PFA-100® uses two different test cartridges containing either collagen plus ADP (CADP cartridge) or collagen plus epinephrine (CEPI cartridge) mimicking vascular endothelium damage and primary platelet clot formation. Within the cartridge, the citrated blood is aspirated at high shear stress through a capillary closed at its end by a collagen membrane defining a microscopic aperture and coated with either ADP or epinephrine. The time taken by platelets to form a plug and occlude the aperture is defined as closure time. The clinical value of PFA-100® closure time in the evaluation of platelet function is not clearly established and its use is mainly restricted to research studies and prospective clinical trials [67]. Although simple and rapid, the PFA-100® remains a point-of-care assay with a low throughput (only two channels) and a relatively large sample volume requirement (0.8 mL per test), which limit its use in preclinical studies to large animal species [68,69].

More recently, other assays based on platelet adhesion under shear stress have become available including the IMPACT: Cone and Plate(Let) Analyzer (Image Analysis Monitoring Platelet Adhesion Cone and Plate Technology) (DiaMed, Cressier, Switzerland) [70] and the global thrombosis test (Montrose Diagnostics Ltd., London, UK) [71]. The evaluation of their clinical value is still in progress, and they might play a role in the future for the assessment of platelet function in patients, in clinical trials, and maybe as well in preclinical studies.

Tests based on viscoelastometry

Thromboelastography (TEG®, Haemoscope, IL, USA) and thromboelastometry (ROTEM®, TEM Innovations, Munich, Germany) are two closely-related methods assessing the hemostasis process as a whole. These assays are based on the analysis of viscoelastic forces in WB during clot formation [72]. In these tests, platelets play all their functional roles in hemostasis including clot formation, clot retraction, and clot lysis. The addition of specific reagents induces the activation of hemostasis through either the intrinsic or extrinsic pathway. Different tests are available depending on the reagent used. For each test, several parameters indicating the different steps of hemostasis are measured. Some of them recognize more specifically platelet function contribution but they do not provide a comprehensive or sensitive evaluation of impaired platelet function [73]. In addition, by the selective activation of extrinsic pathway without or with antiplatelet agents, it is possible to evaluate the extent platelet contribution to clot formation and lysis [74]. The main clinical value of viscoelastometry assays is for the predictive evaluation of postoperative bleeding. Both TEG and ROTEM have been used in animals and particularly in laboratory animal species to monitor hemostasis in experimental studies [75–80]. In nonclinical drug safety assessment, these assays offer the possibility to monitor the different steps of hemostasis using one single test and to evaluate the contribution of a possible drug-induced platelet dysfunction to the whole hemostasis process. Of note, the ROTEM platelet system is a new module recently marketed that can be added to the ROTEM instrument, enabling simultaneous analysis in WB of both thromboelastometry and platelet aggregation using impedance aggregometry.

Exploratory platelet function testing assays

None of the available assays aforementioned provide a robust and reliable approach to assess drug-induced platelet dysfunction in nonclinical toxicity studies. A main limitation of all those tests is the need for a fresh blood sample containing intact functional platelets without the possibility to store the sample for analysis in large batches or to perform retrospective analyses from frozen samples. Another limitation is the lack of standardization of those 'functional' assays especially when used in laboratory animal species. This results in a high variability of the data with the lack of clear indications or guidelines for the correct use of such tests in preclinical studies.

An alternative to platelet function and clot formation assays is to use a soluble marker that will be a surrogate of in vivo platelet activation or aggregation. Indeed, once activated, platelets rapidly release into the blood their granule contents, metabolic molecules, and membrane proteins shed from the platelet surface. Several biomarkers of platelet activation such as P-selectin, CD40L, platelet factor-4, GPIIb/IIIa, GPVI, or thromboxanes have been identified which can be assessed directly in the blood (or in the urine) by robust laboratory methods such as immunoassays [81–84]. However,

there is a lack of comparative studies providing data on sensitivity and specificity of these markers. Furthermore, they are all 'post-events' and not predictive, giving data about events (platelet activation or aggregation) that have already happened.

Another type of exploratory soluble markers of platelet function are represented by platelet-related microRNAs (miRNAs) such as miR-126 and miR-223 that can be readily evaluated by real-time quantitative polymerase chain reactions in PRP, (platelet-poor) plasma, or serum [85–87]. Promising opportunities exist to further pursue platelet miRNAs as predictive biomarkers for platelet (dys)function in preclinical toxicity studies.

In any case, these assays allow the evaluation of the vascular endothelium and the blood flow that are major components of primary hemostasis and participate to platelet function. This major gap is partly overcome by the introduction of new platelet adhesion tests using microfluidic devices, for example, the organ-on-a-chip technology which has been recently adapted to blood vessels (vessel-on-a-chip) with a microengineered on-chip system containing human endothelial cells perfused with human WB at physiologically relevant shear rates [88–90]. Pioneering studies demonstrated the translational potential of this technology to evaluate and predict drug-induced platelet dysfunction and related risk of thrombosis [90].

Recommended approach for assessing platelet function in nonclinical drug safety

Although waiting for the capability to evaluate platelet function more systematically in preclinical toxicity studies as a routine safety biomarker, platelet function should be evaluated with a more flexible and scientific approach. This approach should be driven by a better understanding of platelet biology, drug pharmacology, and their potential interactions. This first step can be performed very early in silico and/or during the in vitro safety pharmacology profiling. At this stage, if a potential platelet–drug interaction is suspected, it should be further investigated first in vitro/ex vivo with spiking experiments assessing the real effect of the drug candidate on platelet function. This can be carried out, for instance, for platelet aggregation with platelet aggregation studies using human blood from healthy volunteers and/or blood from laboratory animal species allowing cross-species comparison. If deemed necessary, the potential drug-related dysfunction should be evaluated in vivo in general or in dedicated investigative preclinical toxicity studies using specific platelet function biomarkers (BTs, WBaggregability, FC-derived platelet activation markers, etc.), and eventually with relevant animal models of bleeding or thrombosis. The possible synergistic effects of the drug candidate with standard antiplatelet and/or anticoagulant drugs should be evaluated as well during the nonclinical safety assessment. The outcome of these nonclinical safety investigations should help to define a safety biomarker strategy for early phase clinical trials including relevant platelet function testing assays and eventually to guide patient selection for the clinical trials excluding, for instance, patients with high risk of bleeding or thrombosis.

Conclusions

Preclinical testing of platelet function during drug development provides pharmaceutical companies an opportunity to identify drug-induced platelet dysfunction and related hemostasis defect that may potentially lead to increased risk of either bleeding or thrombosis in patients. However, in the current practices for evaluating hemostasis in drug development, evaluation of platelets is largely confined to parameters measured by standard hematology analyzers such as the peripheral blood platelet count and indices [7,8], whereas platelet function is usually not evaluated [9]. Thus, although drug-induced platelet dysfunction and related risk of bleeding or thrombosis in patients are a very pertinent safety concern, they are currently poorly and inconsistently evaluated in drug safety causing attrition of drugs most prominently during late-stage clinical trials and postapproval phase of drug development. On the other hand, significant progress has been made in elucidating the molecular mechanisms of platelet function [3,91,92], and in this review article we have shown that methods exist to detect altered platelet function in nonclinical studies. What is still missing is a more informative and effective strategy of platelet function testing in nonclinical drug safety assessment, and its implementation on a pharmaceutical industry–wide basis. Standard platelet function testing assays (platelet aggregometry, FC-based assays) that can be used in laboratory animal species have some limitations including need of specialized laboratory resources (analyzers and technicians), stringent sample management requirements, low throughput, that do not support their systematic use in preclinical toxicology studies as standard safety biomarkers. The development of new innovative platelet function testing assays including, for instance, platelet transcriptomics or proteomics offer a new perspective [93–96]. With these new methods, sample management and analytical procedures are more simple and flexible with, for example, the possibility to work from frozen samples allowing delayed or even retrospective evaluation of platelet function after study completion. However, the performance of these new assays for the detection of drug-induced platelet dysfunction remains to be established, as for the platelet function assays described in this review. Ultimately, the retrospective evaluation of the outcome of implementing platelet function testing assays during drug

development will determine the effectiveness of this strategy for improving patient safety.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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