Platelet function testing in drug discovery projects: considerations and challenges

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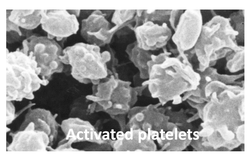
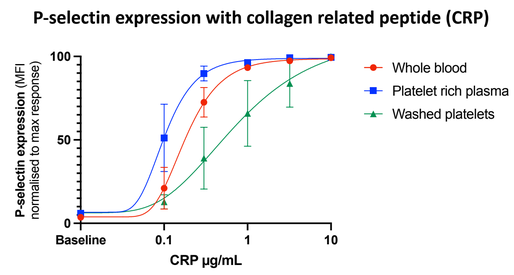
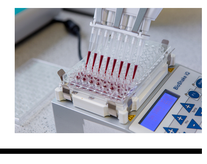
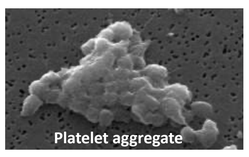
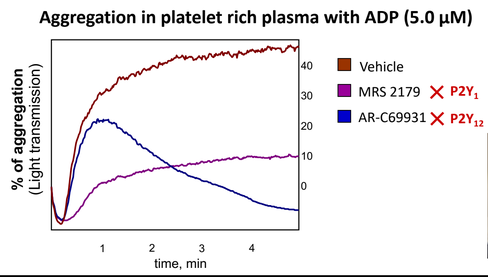
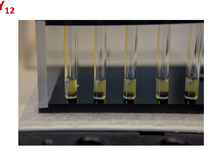
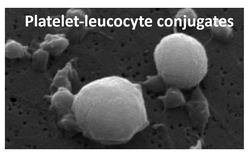
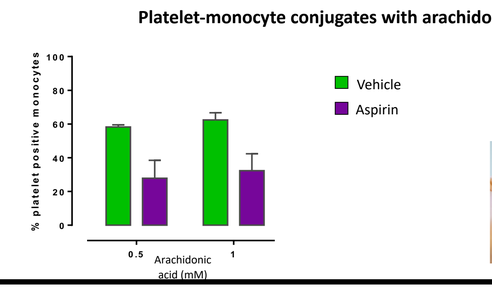
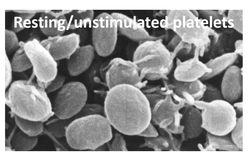
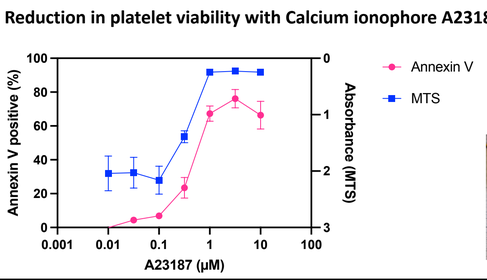
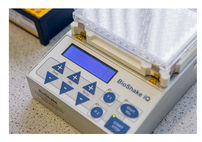
Introduction and Conclusion

Testing for possible effects of agents on platelet function requires consideration of several aspects of platelet physiology and the impact of these on sample viability and study integrity. The pharmacology and characteristics of the test item(s) and the study objective are also key factors in choosing the most appropriate test system. Alignment of project specifics and platelet function testing expertise can ensure design of a tailored, decision-making study.

Challenges

- Since the main physiological role of platelets is to provide primary haemostasis when vessel wall integrity is compromised, the first challenge is to **avoid/minimise platelet stimulation while drawing blood**
- Obtaining a viable sample of sufficient volume from **preclinical species** (primates, dogs, rats) has additional technical challenges
- **Limited window to perform assays** - platelet viability reduces substantially over time, with an optimal window of 4-6 hours post sampling. This also means that any requirement to transport samples can be problematic.

Considerations for choosing the testing matrix and platelet function assay

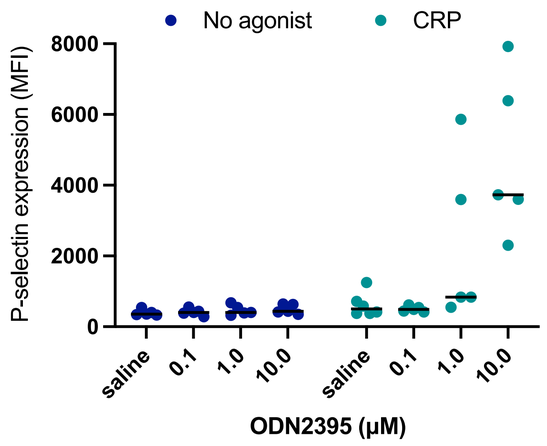
Platelet function assay	Whole blood	Platelet rich plasma	Washed platelets	Assay characteristics and examples
Activation/granule release 	✓ ▪ Flow cytometry ▪ High throughput	✓ ▪ Flow cytometry ▪ High throughput	✓ ▪ Flow cytometry ▪ Relatively high throughput	▪ Assessment of activation markers expression by flow cytometry (e.g., P-selectin, CD63, PAC1 - activated GPIIb/IIIa) ▪ Performed on a 96-well plate and samples are fixed after activation, so the time of analysis can be flexible  
Aggregation 	✓ ▪ Flow cytometry or impedance ▪ Can be high throughput (plate)	✓ ▪ Light transmission or flow cytometry ▪ Can be high throughput (plate)	✓ ▪ Light transmission or flow cytometry ▪ Can be high throughput (plate)	▪ Assessment of light transmission through a stirred or shaken sample of platelet rich plasma over time ▪ Performed in a specialised aggregometer or on a 96-well plate using a plate reader  
Platelet-leucocyte conjugates 	✓ ▪ Flow cytometry ▪ Low throughput ▪ Not used frequently			▪ Assessment of platelet-specific markers on leucocytes ▪ Can be measured within the samples processed and fixed for whole blood platelet aggregation measurement  
Platelet viability/ apoptosis 		✓ ▪ Flow cytometry or light absorbance ▪ High throughput	✓ ▪ Flow cytometry or light absorbance ▪ High throughput	▪ Assessment of phosphatidyl serine (PS) exposure with Annexin V binding or enzymatic activity by MTS assay or cell membrane integrity by lactate dehydrogenase (LDH) release  
Close to physiological	+++	++	+	
Small blood sample volume	+++	++	+	
"Clean" system	+	++	+++	

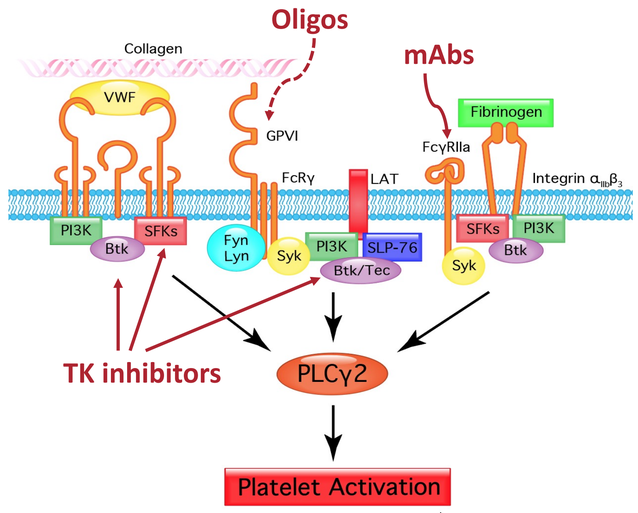
Most common types of agents we have evaluated:

- ✓ Oligonucleotides
- ✓ Monoclonal antibodies
- ✓ Tyrosine kinase inhibitors
- ✓ GPCR antagonists

Oligonucleotides

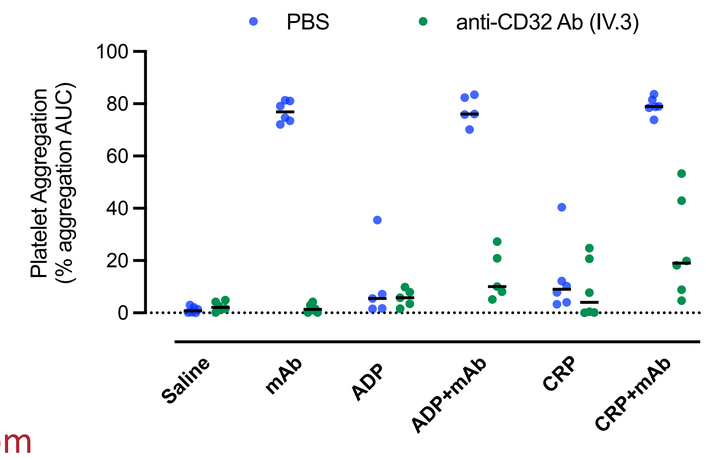
Platelet activation (P-selectin expression) in whole blood with a PS-backbone modified oligonucleotide ODN2395 alone and in combination with a threshold concentration of platelet agonist (CRP)





Monoclonal antibodies

Aggregation in platelet rich plasma with an activating monoclonal antibody (mAb) alone and in combination with threshold concentrations of platelet agonists (ADP and CRP) inhibited by blocking anti-CD32 antibody



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