Application of platelet testing in drug discovery

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Introduction

Platelet function testing has historically been a core component in the discovery and development of novel anti-thrombotic agents designed to target receptors, enzymes or adhesion proteins involved in platelet functional responses. Recent developments in drug discovery have led to various biologics targeting more disease areas that may unexpectedly target platelets, risking side effects of thrombosis or bleeding, depending on whether stimulation or inhibition of platelet function is involved. Platelet function testing has recently highlighted two modalities, monoclonal antibodies (mAbs) and oligonucleotides (ONs), showing their effects on platelet function.

Monoclonal antibodies

Monoclonal antibodies (mAbs) in development are often IgG isotype and have been shown to activate platelets via cross-linking of the platelet FcγRIIA receptors if the target is expressed on platelets or present in circulation[1].

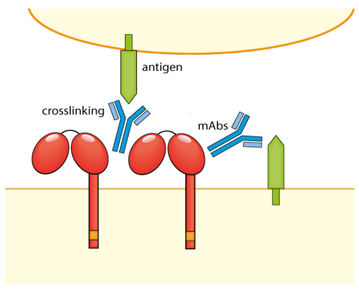


Diagram 1. Crosslinking of FcγRIIA receptors, in clusters on platelets, by a mAbs leading to platelet activation.

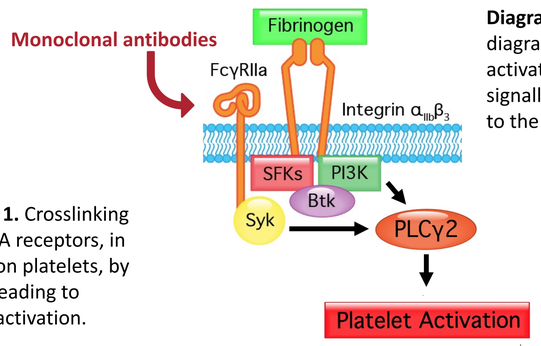


Diagram 2. Schematic diagram of platelet activation through a signalling pathway, linked to the FcγRIIA receptor.

- Anti-CD226 (IgG mAb) directly induced platelet aggregation in a concentration-dependent manner.
- No direct effect of anti-CD226 on platelet activation was observed, however anti-CD226 potentiated CRP-induced platelet activation.

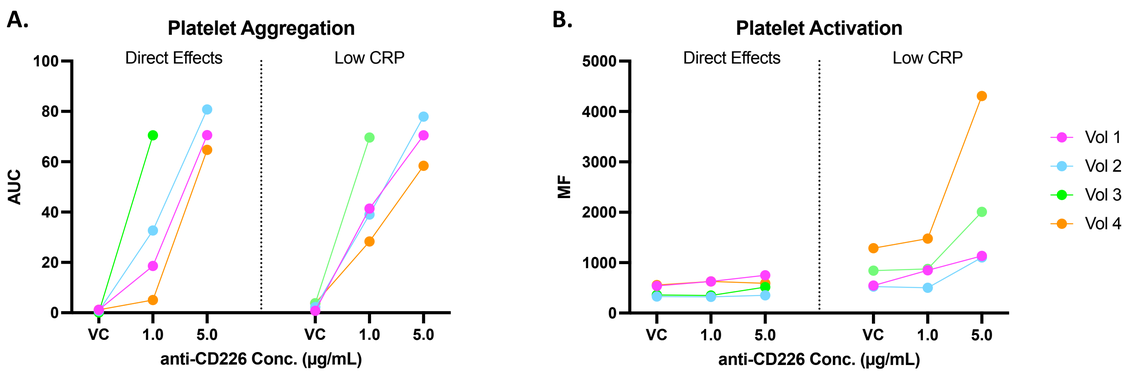


Figure 1. (A) Area under the curve (AUC) was measured in platelet rich plasma (PRP) by light transmission aggregometry (LTA) and (B) median fluorescence (MF) of P-selectin (CD62P) expressed on platelets in whole blood (WB) was measured by flow cytometry (FC). Both assays were performed in the presence of PBS (VC) and anti-CD226 (clone LeoA1) at 1.0 and 5.0 µg/mL without an agonist (direct effects) and with stimulation of platelets by a low CRP concentration (n=4), vol (volunteer).

- Anti-CD32, a blocking antibody that inhibits platelet activation via the FcγRIIA receptor[1], fully inhibited aggregation induced by anti-CD226.

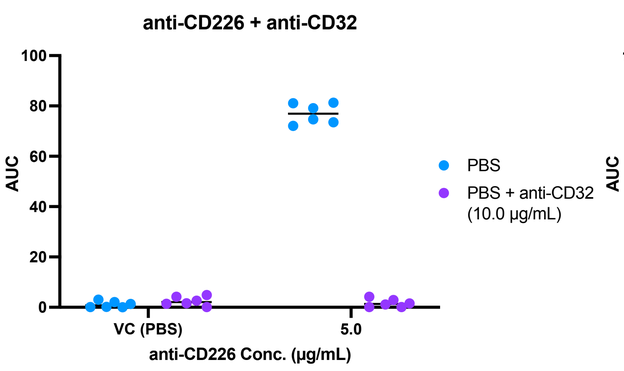


Figure 2. AUC was measured by LTA in PRP with PBS alone and PBS with anti-CD32 (clone AT10) at 10.0 µg/mL in the presence of PBS (VC) or stimulated with anti-CD226 (clone LeoA1) at 5.0 µg/mL (n=6, median).

- Anti-CD226 induced full platelet aggregation in eight volunteers, but no aggregation was observed in four volunteers, possibly due to polymorphisms of the platelet FcγRIIA receptor[2].

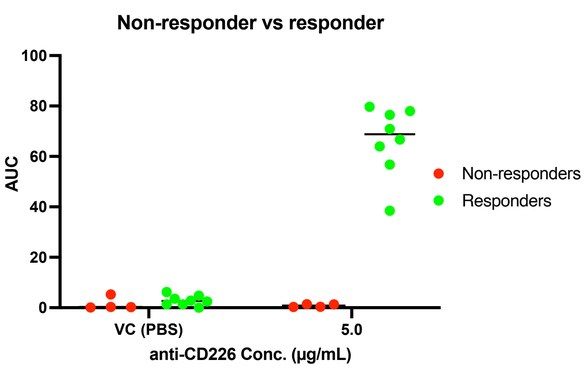
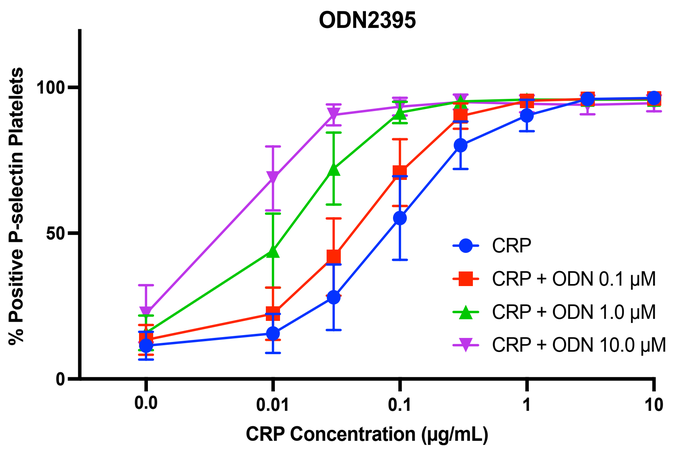


Figure 3. AUC was measured by LTA in PRP with PBS alone and PBS with anti-CD226 (clone LeoA1) at 5.0 µg/mL in "non-responders" and "responders" (n=6, median).

Oligonucleotides

Oligonucleotides (ONs) developed as potential drug candidates can have phosphorothioate (PS) modifications to prevent rapid degradation by nucleases. PS-modified ONs have been shown to elicit platelet activation and platelet aggregation, potentially via the GPVI receptor[3].



- ODN2395 (PS modified ON) potentiated CRP-induced platelet activation in a concentration-dependent manner.
- Slight platelet activation was induced directly by ODN2395 at 10.0 µM.

Figure 4. % positive P-selectin (CD62P) expressed on platelets in WB was measured in the presence of saline (VC) or ODN2395 at 0.1, 1.0 and 10.0 µM stimulated with a range of CRP concentrations.

- At the highest concentration, 10.0 µM, ODN2395 directly induced delayed platelet aggregation in volunteers 1 and 3.
- ODN2395 at 1.0 µM strongly potentiated CRP-induced platelet aggregation in volunteers 1 and 4, but not in volunteers 2 and 3.
- In all volunteers, ODN2395 at 10.0 µM strongly potentiated CRP-induced platelet aggregation.

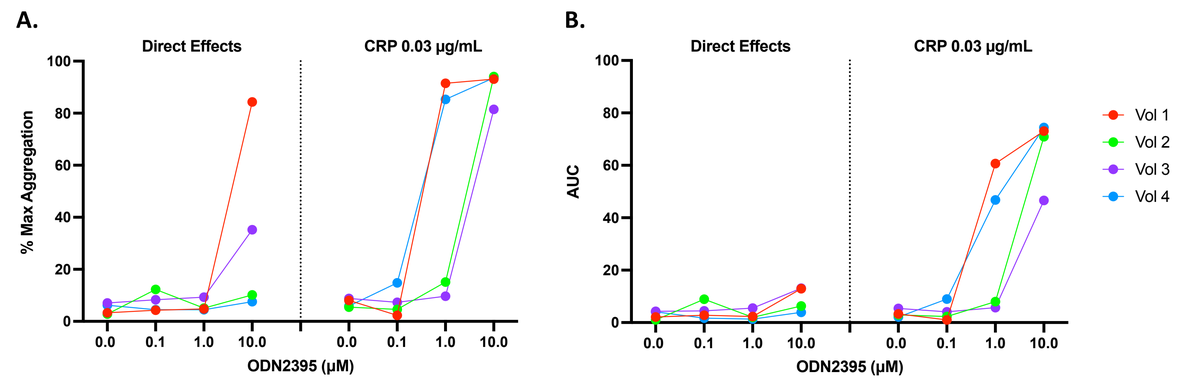
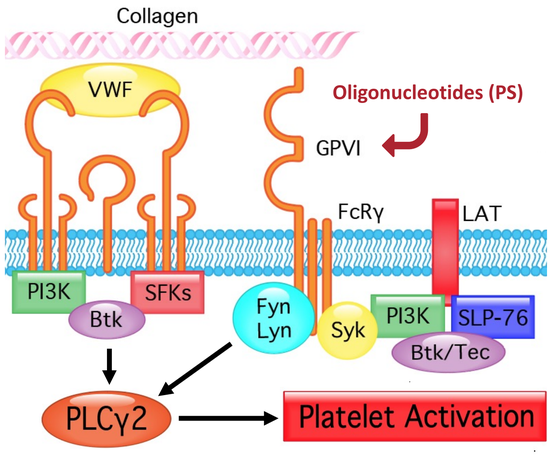


Figure 5. (A) % max aggregation and (B) AUC were measured by LTA in PRP in the presence of saline (VC) or ODN2395 at 0.1, 1.0 and 10.0 µM without an agonist (direct effects) and stimulated with CRP 0.03 µg/mL (n=4), vol (volunteer).



Considerations:

- Clinical thrombocytopenia induced by ONs with PS backbone only occurred in a small number of individuals[4].
- Platelet function testing could be used early in drug discovery to de-risk the ONs in development or as a tool to identify individuals at risk of ONs-induced thrombocytopenia.

Conclusions

- Platelet function testing in early drug discovery projects can help de-risk unexpected thrombosis and bleeding events in toxicology and early clinical testing.
- There is potential to build platelet function testing into screening strategies as part of compounds and biologics selection using higher throughput assays.

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References

1. Arman *et al.,* Human platelet IgG Fc receptor FcγRIIA in immunity and thrombosis. Journal of Thrombosis and Haemostasis. (2014) 13, 893-908.

2. Chen *et al.,* Platelet FcγRIIA HIS131ARG polymorphism and platelet function: antibodies to platelet-bound fibrinogen induce platelet activation. Journal of Thrombosis and Haemostasis. (2002) 1, 355-362.

3. Flierl et al., Phosphorothioate backbone modifications of nucleotide-based drugs are potent platelet activators. The Journal of Experimental Medicine. (2015) 212(2), 129-137.

4. Slingsby *et al.,* Sequence-specific-2'-O-methoxyethyl antisense oligonucleotides activate human platelets through glycoprotein VI, triggering formation of platelet-leukocyte aggregates. (2022) 107(2), 519-531.