Assessment *in vitro* of potential effects of therapeutic oligonucleotides on platelet function

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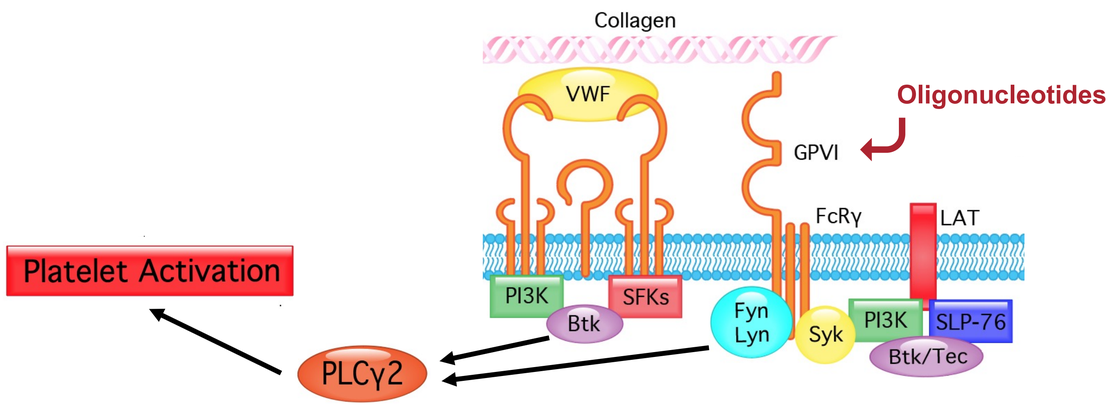
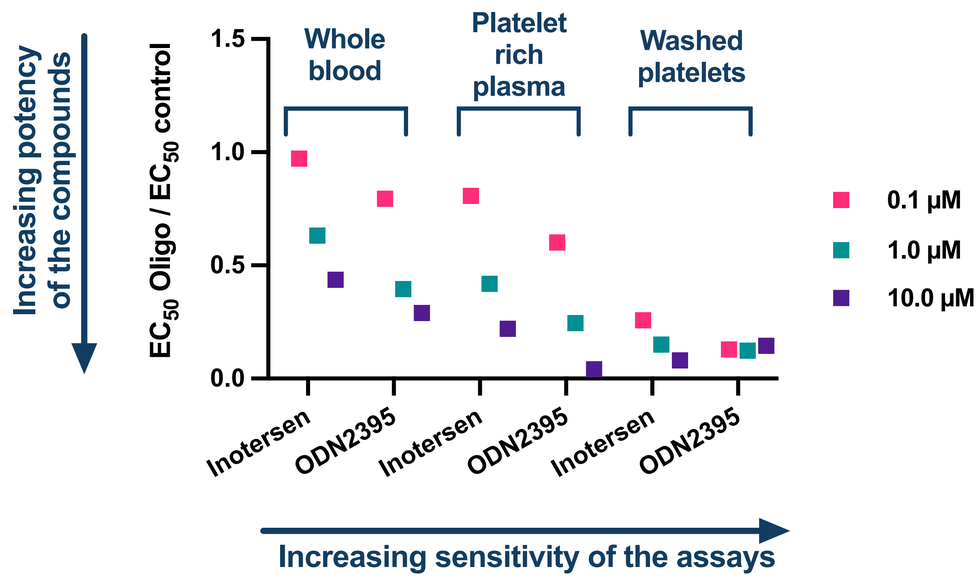
Background

Multiple antisense oligonucleotides (ASOs) have been developed and some approved for various indications. However, in toxicological assessment and clinical studies a dose- and sequence- dependent thrombocytopenia (TP) of varied severity has been reported (1). This has been mainly attributed to pro-inflammatory and platelet activating effects of the ASOs (2,3), leading to enhanced platelet sequestration. Accordingly, access to reliable and high throughput platelet function assays would aid safety assessment of ASOs earlier in development.



Conclusion

Platelet function testing can be used to de-risk selection and progression, or as a tool to identify individuals at risk of ASO-induced TP. Availability of a range of sensitive, reproducible, plate-based test systems allows platelet function testing to be considered as part of a screening strategy.



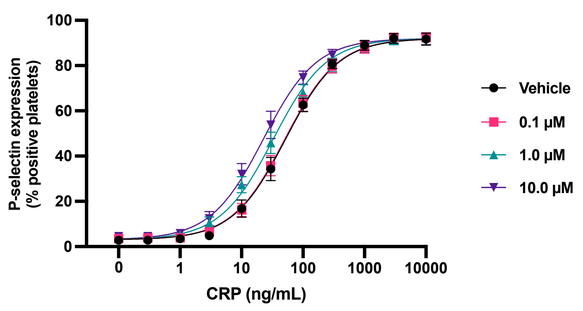
Test Systems & Results

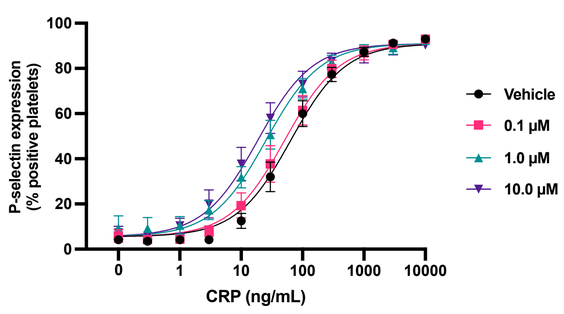
Effects of the reference compounds, inotersen and ODN2395, were assessed using two different assays in three matrices, directly and in combination with collagen related peptide (CRP), a GPVI receptor agonist (mean ± SEM, n=5-6). All three assays showed an increase in potency of CRP in the presence of ODN2395 and inotersen; the sensitivity of the assays to demonstrate the effects of the two ASOs was compared by quantifying the shift in EC_{50} in the presence of three concentrations of each compound.

Inotersen:
produced TP in toxicology and clinical studies

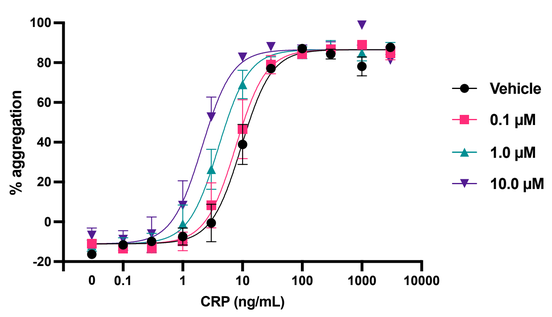
ODN2395:
known to stimulate platelets *in vitro*

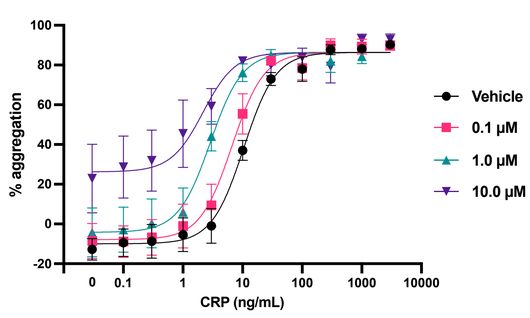
Platelet activation
- whole blood
- flow cytometry



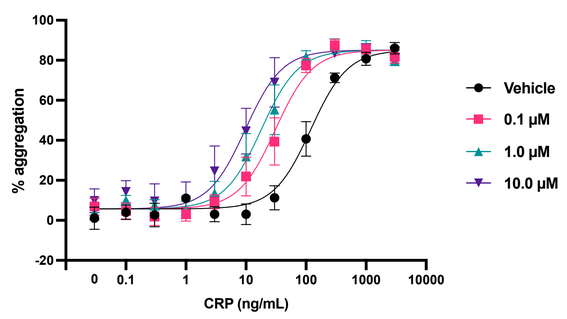


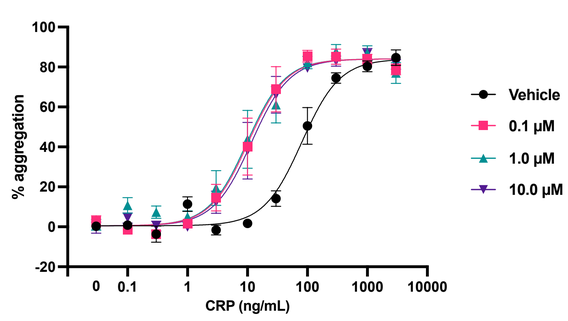
Platelet aggregation
- platelet rich plasma
- light absorbance





Platelet aggregation
- washed platelets
- light absorbance



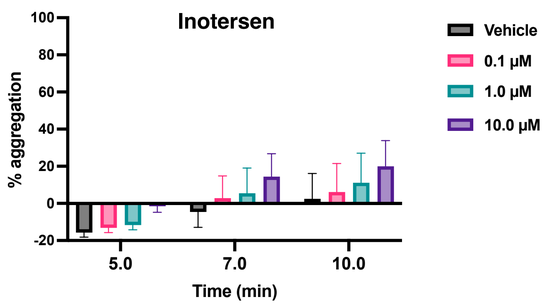


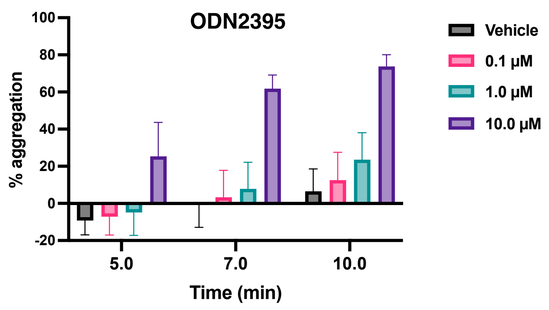
The least sensitive **platelet activation assay** can be applied to testing *ex vivo* in samples from pre-clinical and clinical studies of ASOs, since it is performed in **whole blood** on fixed samples.

Only **aggregation in platelet rich plasma** showed direct effects of the two ASOs on platelet aggregation at 5 minutes (graphs above) and later time-points (graphs below)

Aggregation in washed platelets provides the most sensitive assay to investigate the effects of ASOs on platelets

Direct effect of ASOs on platelet aggregation in platelet rich plasma:





References:
(1) The Effects of 2′-O-Methoxyethyl Containing Antisense Oligonucleotides on Platelets in Human Clinical Trials (Crooke et al., 2017).
(2) Phosphorothioate backbone modifications of nucleotide-based drugs are potent platelet activators (Flierl et al., 2015).
(3) Assessing single-stranded oligonucleotide drug-induced effects in vitro reveals key risk factors for thrombocytopenia (Sewing et al., 2017).