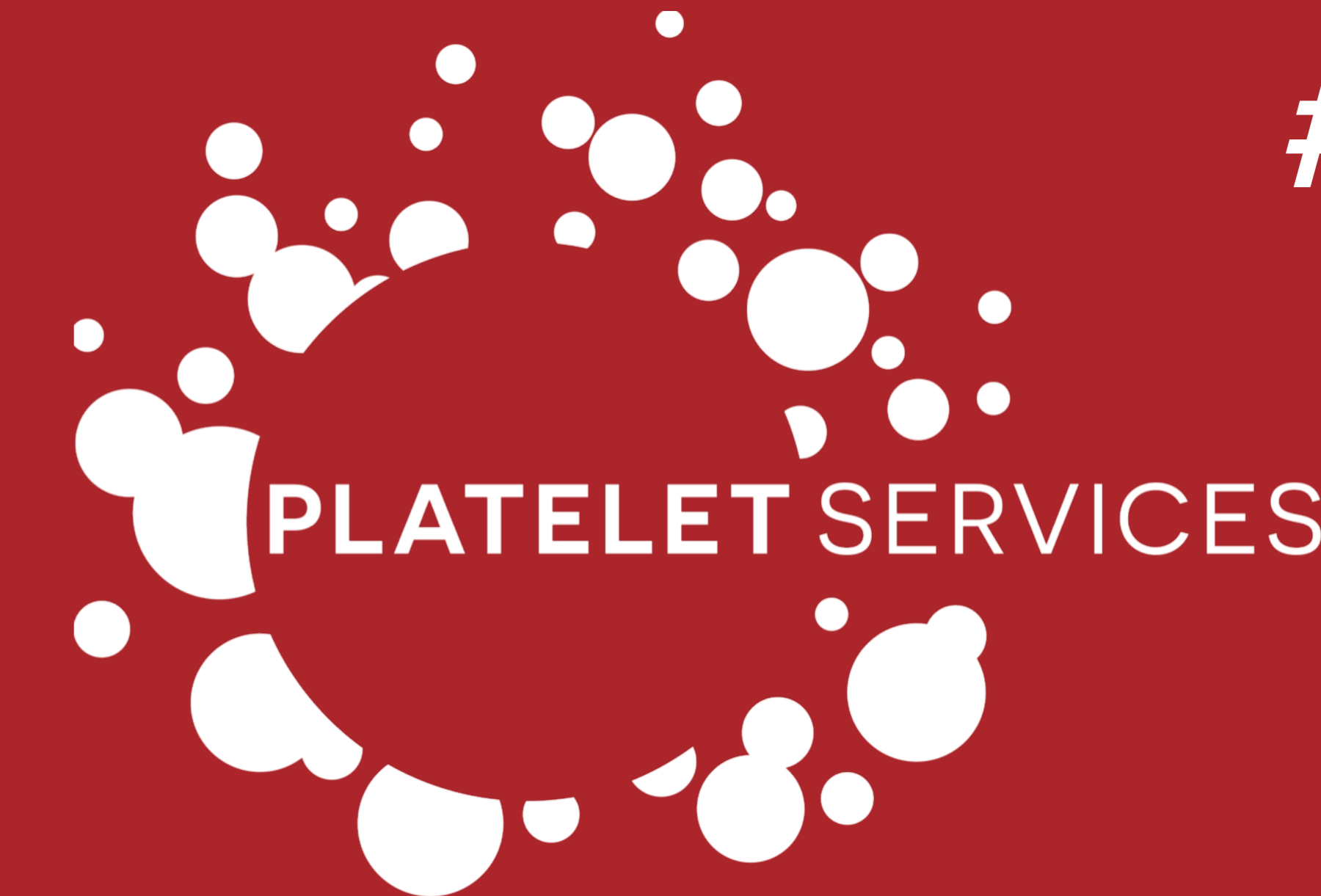


Off-Target Platelet Sensitisation by Therapeutic Oligonucleotides: Validation of a High-Throughput Screening Approach

#3530



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Background

Synthetic oligonucleotides (ONs) are increasingly recognised to exert unintended effects on platelets. Several phosphorothioate-modified ONs have been shown to enhance platelet activation, aggregation, or clearance through off-target interactions, raising concerns regarding potential thrombotic liability. Clinically relevant example, inotersen, was previously shown to potentiate platelet activation. Our earlier work demonstrated that collagen-related peptide (CRP) potency is enhanced in washed platelet aggregation assays in the presence of inotersen and ODN2395, a CpG-rich ON. However, this effect can reach a plateau, requiring multiple concentrations for accurate potency assessment, thereby limiting assay throughput.

Objective

To develop and validate a higher-throughput method that would be suitable for screening a larger number of ONs and would be capable of quantifying off-target platelet sensitisation. In the current study we have used and assessed:

- Tool ONs – ODN2395, ODN2395-PO, inotersen, inotersen-neg and ISIS120704
- A single subthreshold CRP concentration (selected individually for each volunteer/experiment)
- A concentration-response curve for each oligonucleotide, and
- Pooled washed platelets from two volunteers to increase assay throughput.

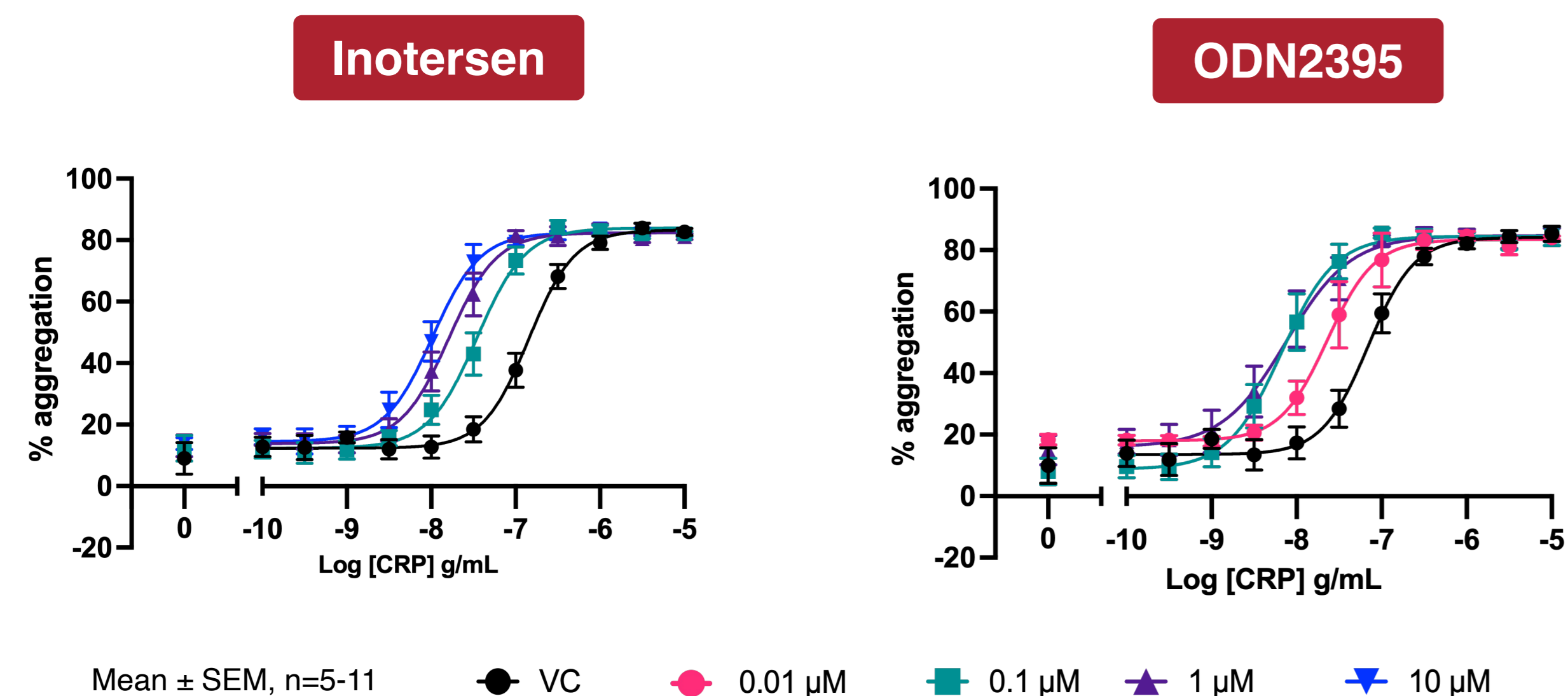
Conclusion

- Oligonucleotide-induced platelet sensitisation is not concentration dependent meaning pS_2 measurements with a single concentration of ONs are not suitable as a screening metric.
- A modified assay format **using one subthreshold concentration of CRP and a full ONs concentration-response curve** reliably generates pEC_{50} values for higher-throughput screening.
- **Pooling platelets** from two volunteers increases throughput without compromising sensitivity or reproducibility.
- This validated platform **enables efficient screening of larger oligonucleotide libraries (up to 80 ONs)** to identify platelet-related off-target effects.

1

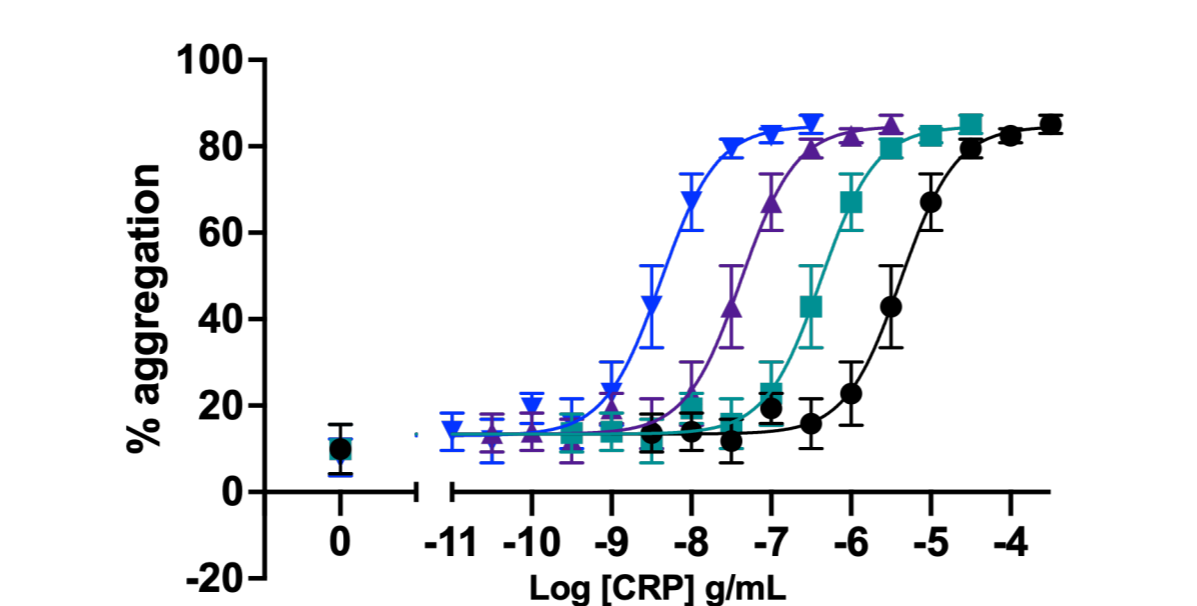
Results

The effects of the **ODN2395** and **inotersen** were assessed in **human washed platelets aggregation** assay (measured as changes in light absorbance over time) in the presence of a 12-point concentration response curve of CRP (collagen related peptide, GPVI agonist).



- Both inotersen and ODN2395 increased the potency of CRP
- The effect plateaued for ODN2395 at 0.1 μ M
- This approach can demonstrate both negative and positive modulation
- Limitation – up to 8 molecules can be assessed per volunteer; not suitable for screening large numbers of molecules

- Assessing CRP potency shift with a single ONs concentration would increase the assay throughput.
- However, inotersen and ODN2395 enhanced CRP potency (graphs above) not in a concentration dependent manner, resulting in different pS_2 (potency-shift-2-fold) values at each concentration (table).
- In contrast, simulated small-molecule data (right graph), show a concentration-dependent leftward shift of CRP response curve, producing a consistent pS_2 value across all concentrations.
- Therefore, a single concentration pS_2 approach cannot be used reliably to screen oligonucleotides.

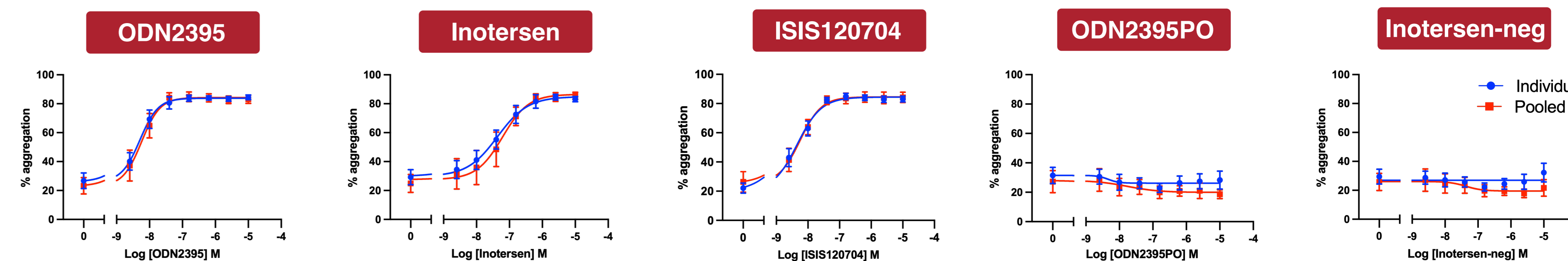


pS_2 (Log M)	Concentration (μ M)			
Compound	0.01	0.1	1	10
ODN2395	8.3	8.0	6.9	
Inotersen		7.5	6.9	6.1
Simulated data		8.0	8.0	8.0

2

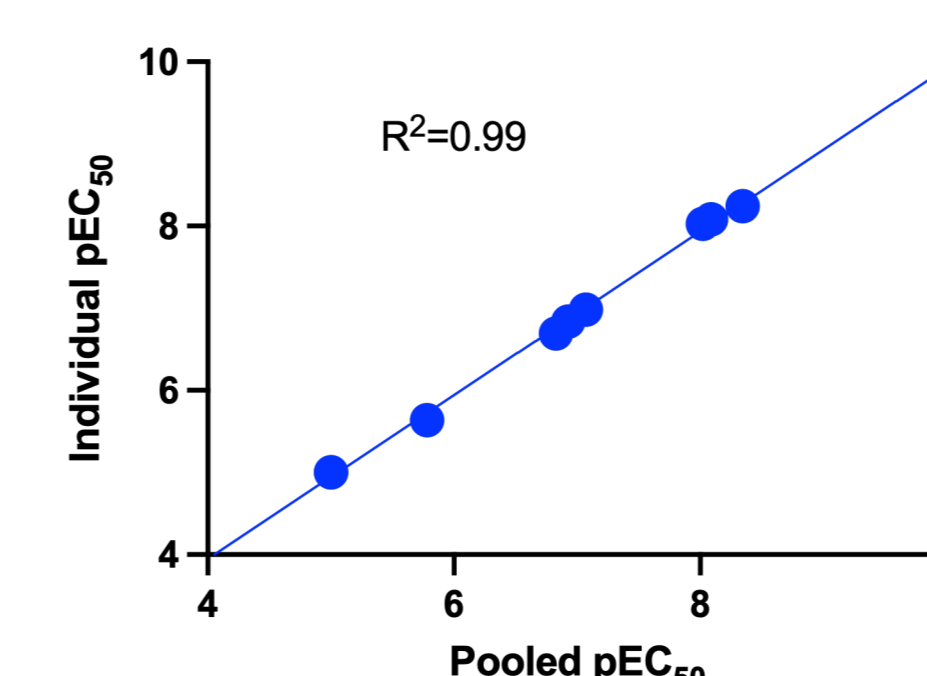
Results

An alternative approach was developed using a single subthreshold concentration of CRP with a wider range of ONs concentrations to generate a pEC_{50} for **ODN2395** (phosphorothioate CpG ODN), **ODN2395PO** (phosphodiester bonds), **inotersen**, **inotersen-neg** (phosphodiester bonds), and **ISIS120704** in individual and pooled samples.

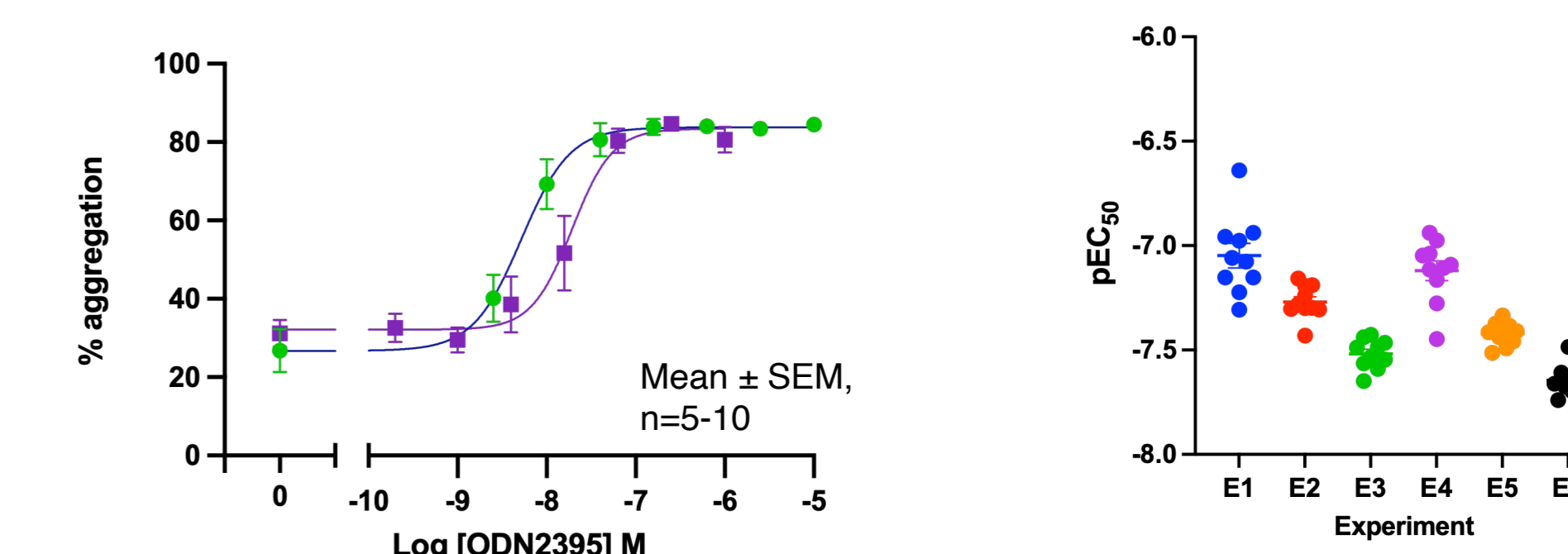


Mean \pm SEM, n=10 and 5 (pooled); CRP concentration was selected in individual samples based on the maximum fold increase in aggregation with ODN2395

- The same relative potency of platelet sensitization by ONs was observed using curve shift (Results 1) and pEC_{50} values.



- To further **increase assay throughput** (up to 80 ONs) we compared the pEC_{50} values obtained in washed platelets pooled from two volunteers and in individual volunteers, observing good correlation.



- Good **inter-assay** and **intra-assay variability** was achieved: graphs are showing aggregation curves with ODN2395 obtained in two different studies (left graph) and pEC_{50} values obtained on 10 plates within each of the 6 experiments (right graph).