Immunogenicity Assessment for an Antisense Oligonucleotide

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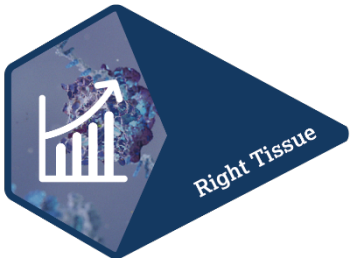
Why Oligonucleotide Therapeutics?

Identifying the **right target**



Making sure our molecule gets to the **right tissue** where it is needed

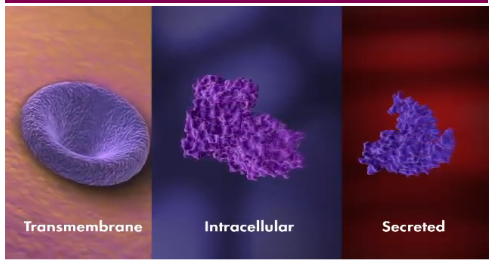
Ensuring **right safety** with minimal side effects

Selecting the **right patients** that will benefit

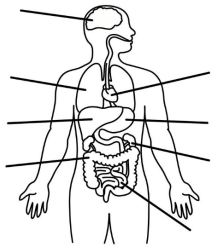
Defining the **right commercial** value and future viability

Underpinned by the **right culture** of truth-seeking behaviours and scientific rigor

Potential to target any type of protein



Potential to target any region of body



Potential to target any disease area

Anti-Sense Oligonucleotide Therapeutics (ASO)

ASO ≠ small molecules

Size

Distribution

Species selectivity

Selectivity

Charge

Immunogenicity

Mechanism of action

Metabolism

$t_{1/2}$

Uptake mechanism

Same development principles but…

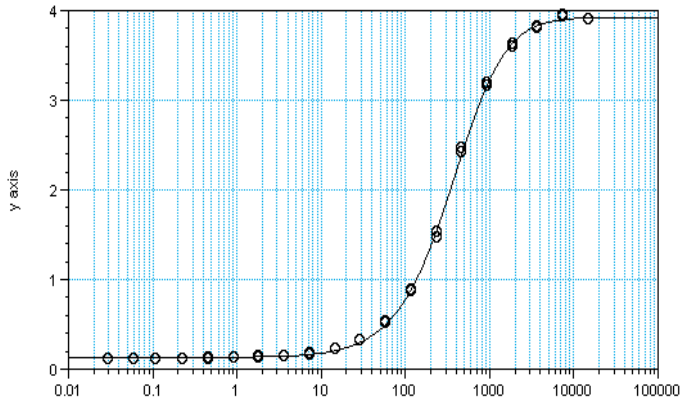
- Different screening cascades
- Different focus areas
- Different approaches

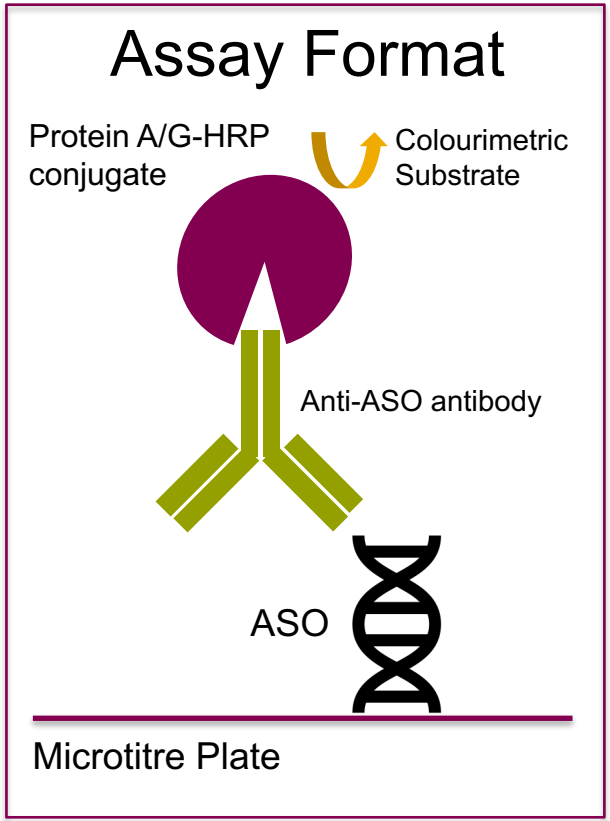
ASO ≠ Biologics

- Regulatory landscape states “case by case” approach for oligonucleotide therapeutics with a focus on clinical
  - Limited guidance for nonclinical studies

Anti-Immunogenicity Assay

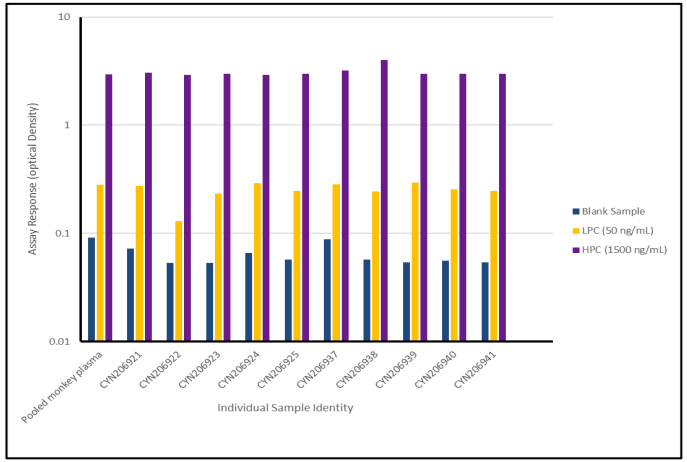
- Sequential format direct assay
- Anti-ASO antibodies are captured to ASO immobilised on a microtitre plate
- Bound antibodies are detected using Protein A/G-HRP conjugate
- Proportional relationship between amount of ADA and assay response
- No evidence of prozone effect

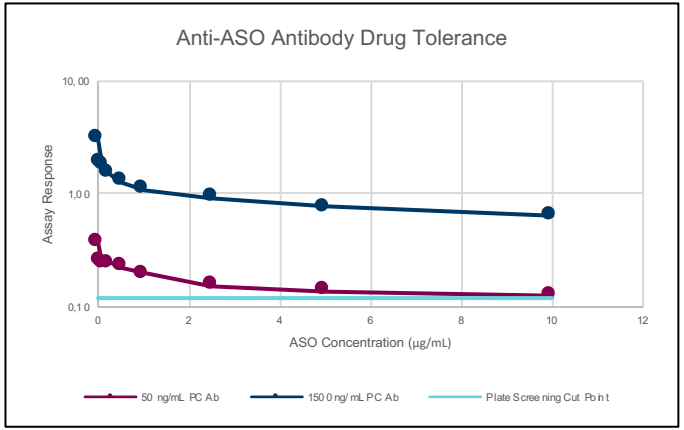




Non-Clinical ADA Assay Validation

- Extended analysis for this example to build knowledge of assay behaviour
  - Screening, Confirmatory and Titer assays conducted
  - 3 Tier strategy not recommended for non-clinical studies
- 2 analysts x 2 plates crossover design
- Precision assessment for all three tiers
- Assay Sensitivity <10 ng/mL
- Drug Tolerance up to 10 µg/mL
- Short term stability to cover handling of positive controls
- No long term frozen storage stability



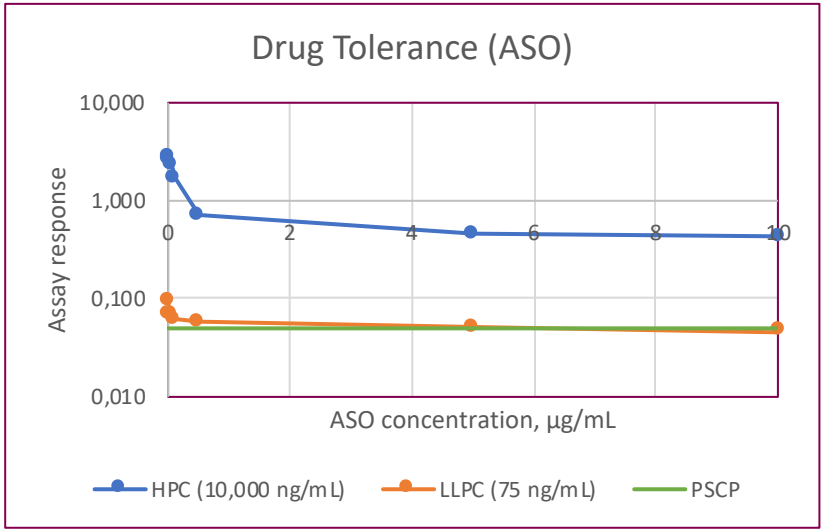


Clinical Immunogenicity Assay Validation

- Screening, Confirmatory, Titration assay
- Validated in accordance with regulatory (FDA/EMA) guidance in both normal human plasma as well as different disease state plasma
- Assay sensitivity ~ 30 ng/mL

- Assay drug tolerance
  - ≤ 20 µg/mL, at HPC and MPC levels
  - ≤ 0.5 µg/mL, at LPC and LLPC levels
  - ≤ expected C_{max} for the two mAb combination products

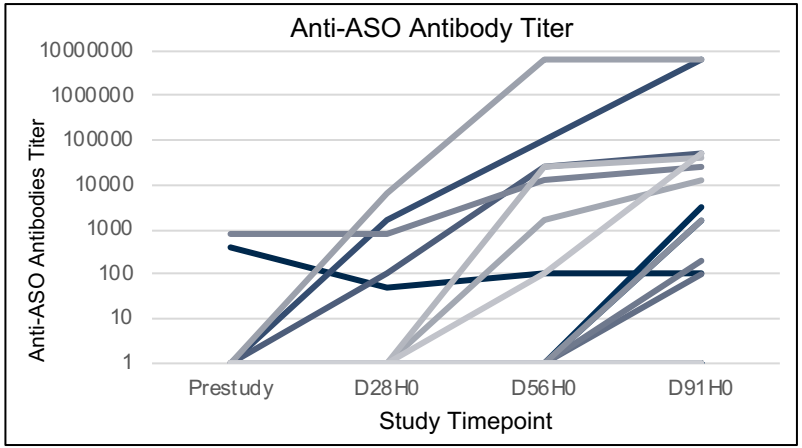
- 6 F/T cycles and benchtop stability up to 29 h at RT confirmed

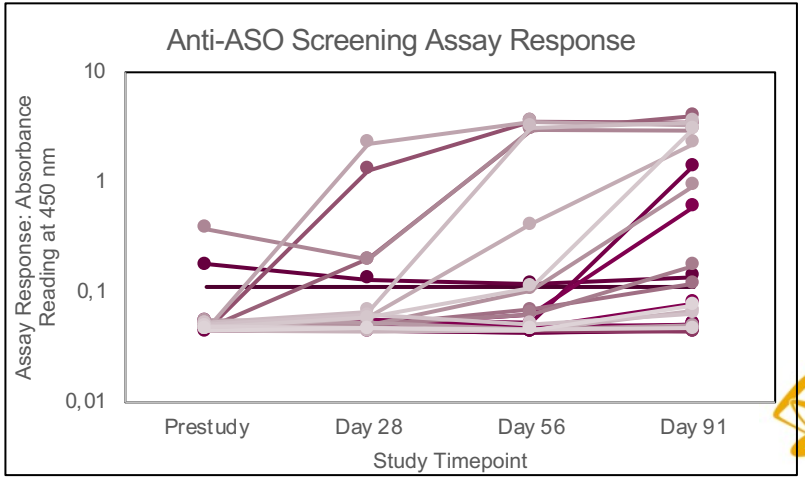


Example Non-clinical immunogenicity response to an ASO

- 50% of ASO dosed animals ADA positive by end of study
- Later onset in lower doses
- Increasing titer with increasing dose
- Confirmatory and Titer data doesn't add substantial value over screening assay response

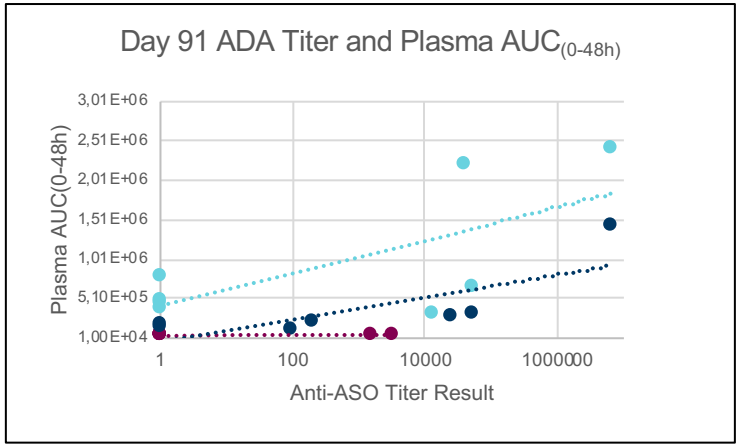
Group	Dose	n	# ADA Positive Animals	# ADA Negative Animals
1	Control	8	1	7
2	3 mg/kg	8	2	6
3	10 mg/kg	8	6	2
4	20 mg/kg	8	4	4
2, 3 and 4	N/A	24	12	12

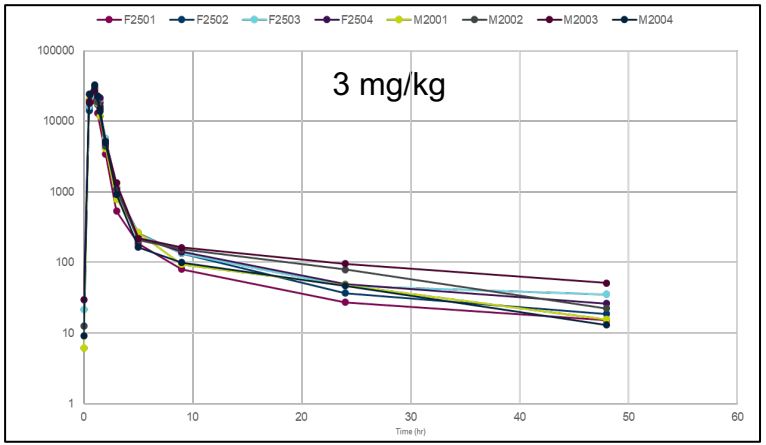


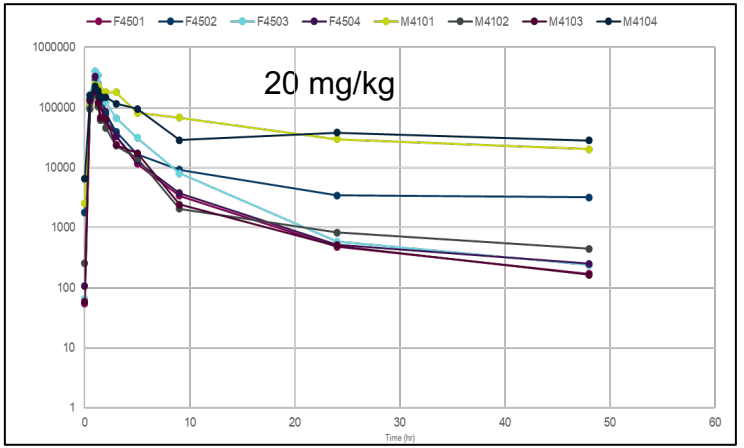


Impact of ADA on Plasma Concentration vs Time

- Plasma Concentration determined using Hybridisation ELISA
- Low dose profiles similar on Day 1 and at End of Study
- Substantial change in distribution phase for in ADA positive animals in high dose group
- Elevated plasma trough concentrations in ADA positive animals
- ADA positive results associated with elevated plasma AUC and increased plasma trough concentration without an impact on C_{max}

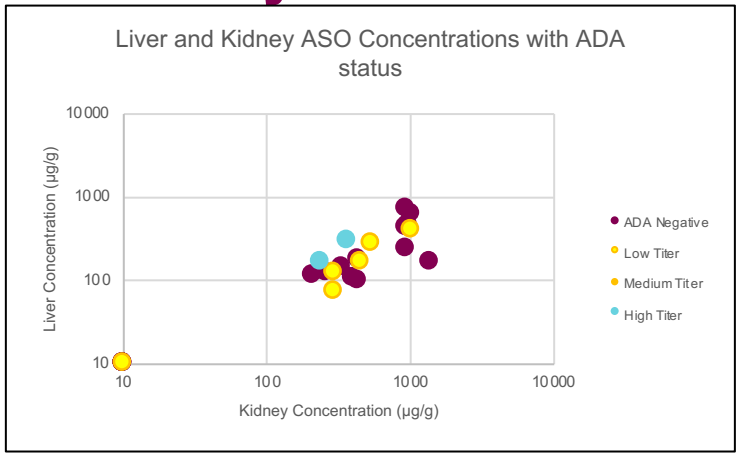


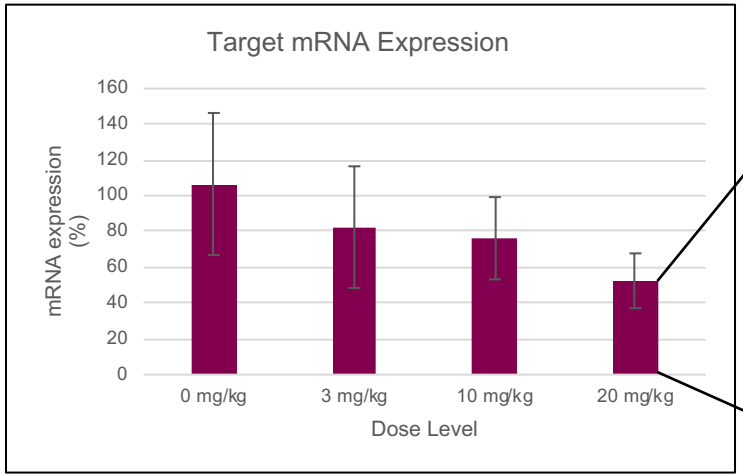


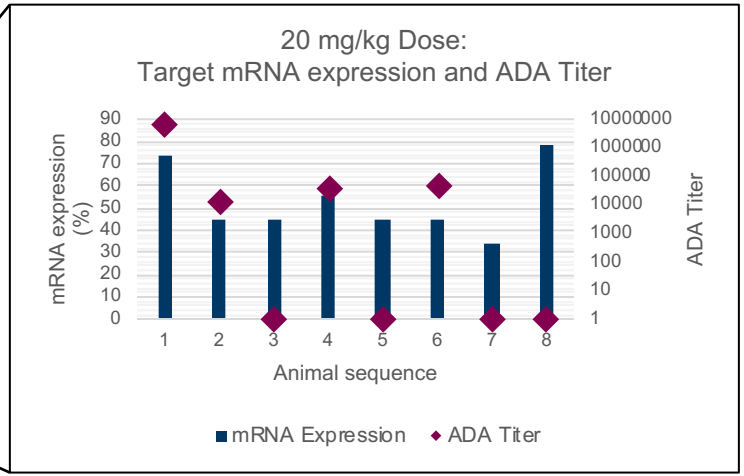


Impact of ADA on exposure and efficacy

- Impact on plasma AUC and tissue exposure only at the very highest anti-ASO titers
- No correlation between ADA titer and mRNA knockdown
- Further supported by other PD analysis





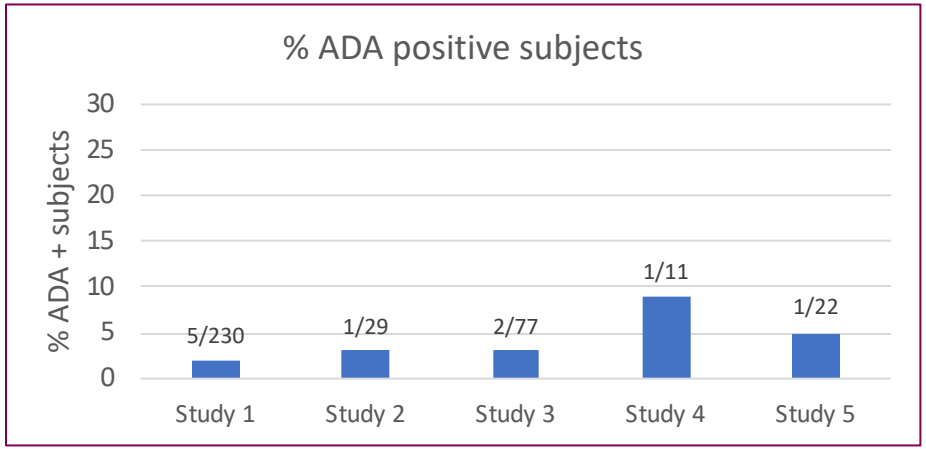


Clinical ADA experience

- The anti-ASO Ab assay has so far been used to support 5 completed clinical studies and provided data from >1000 samples collected from ~350 patients

- The onset of ADAs is usually observed after 6-12 month dosing

  - Only a low incidence of ADA positive (< 10%) subjects have been confirmed in all completed studies



- All current ADA data for the ASO suggest that administration of the ASO possess a low immunogenicity risk without any impact on PK, PD or safety.

Regulatory Expectations and Feedback

- Regulatory expectations and feedback received so far to our clinical protocols recommends;
  - Samples confirmed ADA positive to be titered and evaluated for neutralizing antibodies
  - Unscheduled samples for ADA analysis to be collected in response to suspected immune-related adverse events.
  - Analysis of adverse events that correlate temporally with onset of a positive ADA result at any timepoint after Day 1
  - Patients with treatment-emergent ADA should be followed until the ADA titers have returned to baseline or to a pre-defined low titer

Building the immunogenicity strategy

- An immunogenicity risk assessment to be performed in the early stages of program development – to then evolve with the program as more evidence is collected indicating an increased/diminished risk of immunogenicity.

- The overall immunogenicity risk assessment to guide the clinical monitoring strategy:
  - ADA samples collected and banked
  - ADA samples collected and analysed as part of the clinical study evaluation

- Push back on a dedicated neutralization assay
  - cell-based/competition assays are not relevant since ASOs bind to their RNA substrate within cells
  - if the RNA target produces a soluble protein any ADAs' neutralizing potential is best evaluated by assessing the protein concentration before and after ADA onset.

Conclusions

- Cross-species translation of ASO immunogenicity is not strong
  - Build knowledge initially
  - Limit future non-clinical ADA assessments to screening
  - Consider non-clinical ADA analysis only to answer a specific question
- Overall relatively low clinical immunogenicity risk
- Do not assume the same strategy for ASO as for Biologics
  - ASO immunogenicity may not develop in the same way
  - Impact on PK may be different

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Questions?

Confidentiality Notice