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
- Transmembrane
- Intracellular
- Secreted

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
- Immunogenicity
- Mechanism of action
- Uptake mechanism
- Charge
- Metabolism
- $t_{1/2}$

**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
  – \( \leq 20 \, \mu g/mL \), at HPC and MPC levels
  – \( \leq 0.5 \, \mu g/mL \), at LPC and LLPC levels
  – \( \leq \) expected \( C_{\text{max}} \) 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

<table>
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<th># ADA Negative Animals</th>
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</tbody>
</table>

Anti-ASO Antibody Titer

Anti-ASO Screening Assay Response
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 $C_{\text{max}}$

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Day 91 ADA Titer and Plasma AUC$(_{0-48h})$

Plasma AUC$(_{0-48h})$

Anti-ASO Titer Result

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Plasma Trough Concentration

- 3 mg/kg
- 20 mg/kg
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.

  ![Graph showing % ADA positive subjects across different studies](image)

• 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?
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