Immunogenicity of Synthetic Oligonucleotide: ADA and anti-dsDNA

Crystal Sung, Ph.D., D(ABMLI)
Sr. Scientific Director, Clinical Diagnostics, Biomarkers and Clinical Bioanalyses, TMED
Sanofi R&D
Outline

Immunogenicity of Biotherapeutics

Factors for consideration

Oligonucleotide Therapeutics

Do the same principles apply?

Case Example (ADA and anti-dsDNA)
Why Assess Immunogenicity?

- Aspect of biopharmaceutical clinical trials
  - Anti-drug antibodies (ADA) shown to occur with all product classes
  - Extent of immune responses will be product, indication and treatment-regimen dependent
- Health Authority requirement as part of product safety profile determination
- Increasing evidence that immune responses to biologics can have safety consequences for patients
Why is Immunogenicity Triggered?

- **Neo antigens**
  - Microbial or animal origin/sequences

- **Novel epitopes**
  - Conformational changes in protein, linker region, receptor-Ig chimera

- **Humanized Abs** - anti-idiotype

- Not equally tolerant to all self proteins, particularly if in low abundance

- Immune tolerance breakdown

---

**Immune system has evolved to recognize and eliminate foreign or non-self antigens**
### Factors that Affect Immunogenicity

<table>
<thead>
<tr>
<th>Product Factors</th>
<th>Clinical Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Process</strong></td>
<td><strong>Dose</strong></td>
</tr>
<tr>
<td>- Structural modifications (e.g. oxidation, deamidation)</td>
<td>- Increases with higher doses</td>
</tr>
<tr>
<td>- Impurities that co-purify with the product</td>
<td>- Frequency of administration</td>
</tr>
<tr>
<td>- Contaminants introduced</td>
<td>- Duration of exposure</td>
</tr>
<tr>
<td>- Aggregation; particles</td>
<td>- SC or ID &gt;IM&gt;IV</td>
</tr>
<tr>
<td><strong>Stability</strong></td>
<td><strong>Patient Status</strong></td>
</tr>
<tr>
<td>- Formulation</td>
<td>- Disease status</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>- Immune status</td>
</tr>
<tr>
<td>- Degradation</td>
<td>- HLA background</td>
</tr>
<tr>
<td>- Conformational changes</td>
<td>- Genetic background</td>
</tr>
<tr>
<td>- Aggregation</td>
<td>- Concomitant medication</td>
</tr>
</tbody>
</table>
Immuneogenicity Risk Assessment

\[
\text{Risk} = \text{Severity} \times \text{Probability}
\]

- Consequences of ADAs (Primary Risk Factors)
- Incidence of ADAs (Secondary Risk Factors)
  - Impact on safety
  - Impact on efficacy

ADA Detectability

Measuring ADAs (sensitive and specific assays)

Can pose challenges

LOW/MEDIUM/HIGH risk molecules defined by the potential severity of the clinical consequences linked to the presence of ADAs
Clinical Consequences of Immunogenicity Can Range from Benign to Life Threatening

Immunogenicity Risk Assessment

Impact of ADA:
- No apparent consequence
- Altered PK/PD
- Loss of efficacy
- Safety concerns
- Patient tolerability

Anaphylaxis
Endogenous Cross-reactivity
Immune Complex Deposition – Serum Sickness
Altered PD/Efficacy and Drug Neutralization
Altered PK and Drug Exposure
Injection Site Reactions/ Local Hypersensitivity
Typical Antibody Response Kinetics

- During a primary response, IgM Ab is generally detected first, reaches a maximum in 1-2 weeks and declines within 2-3 months.
- IgG Ab persists for a longer period.
- In a secondary response, IgG Ab rapidly increases.
- Repeat exposure results in:
  - Isotype switching
  - Changes in IgG antibodies over time
    - Increase titers
    - Affinity maturation
    - Epitope spreading
    - IgG subclass changes

![Graph showing typical antibody response kinetics](image)
Overview of Antisense Oligonucleotide

- Highly Specific
- Broad Applicability
- Efficient Development
- Rational Design
- Manufacturing Costs Low
Structure of Representative 2nd Generation Antisense Drug
(A Short Synthetic Chemically Modified Nucleic Acid Polymer)

- ‘Gapmer’ design
  (to activate RNase H)
  - MOE modification at ends
  - DNA in middle
  - Phosphorothioate throughout

- Molecular model of ASO

MOE side chain
DNA/RNA moieties
Phosphorothioate linkage

MOE
DNA
MOE
Can Oligonucleotide Therapeutics Be Immunogenic?

- **Challenges in delivery**
  - Oligonucleotides have poor stability in blood and are readily degraded by nucleases
  - Chemical modifications to minimize exo- and endonuclease digestion improves stability and delivery

- **Modifications may facilitate recognition by the immune system**
  - Incorporate unique structures: sugar residues and inter-nucleotide linkages
  - Prolonged exposure in circulation
  - Immunologic presentation as a hapten via bound to carrier protein, eg. HSA
Antibodies Can Be Elicited Against DNA

Literature Reports (Stollar, FASEB J. 1994; Stollar, Crit Rev Biochem and Molec Bio)

- Anti-DNA Ab could also be induced by exogenous nucleic acid-protein complex
  - Several reports on DNA binding to protein/peptide and being an immunogen
  - Non-immunogenic structure may become immunogenic when forms complex with serum components, aggregates

- Anti-DNA Ab can be generated to modified nucleic acids: conformational or helical structure changes
  - Majority of Ab formed recognize primarily the difference between the immunogen and native DNA
Literature Reports

Anti-DNA Antibodies in SLE

- Patients (40-60%) with active SLE has anti-ds DNA Ab
- Most of SLE patients have Ab that binds to ssDNA
- Ab-DNA complex isolated from damaged kidney in SLE patients (J. Immunol. 1994)
- Drug induced lupus?
Therapies that induce formation of antibodies to dsDNA: Review of US labeling

- Monoclonal Ab products
  - Five TNFα blockers (Humira, Remicade, Enbrel, Cimzia, Simponi) have reported evidence of inducing anti-dsDNA in patients.
  - Some of these patients have developed new autoimmune disease symptoms.
  - Reports of lupus and lupus-like syndromes remain unknown.

- Small molecule therapies
  - (Isoniazid, Hydralazine, etc) historically associated with drug induced lupus are rarely linked to isolated reports of dsDNA antibody.

- Antibiotics
  - Minocycline, has evidence of inducing anti-dsDNA in patients following prolonged treatment. US labeling includes a precaution for auto-immune syndromes.)
Case Example
Familial Hypercholesterolemia

Rare genetic disorder in which both LDL-receptor-alleles are defective

- **Autosomal co-dominant disorder**
  - Homozygotes and Heterozygotes both exhibit disease
  - Heterozygous FH (HeFH) occurring in 1:500 people
  - Homozygous FH (HoFH) affecting 1:1,000,000 people

- **Increased frequency of FH:**
  - Afrikaners of South Africa, French Canadians, Finns, and Christian Lebanese

- **Very high concentrations of LDL and cholesterol in plasma**
  - Greater than 300 mg/dL total Cholesterol in adults
  - Greater than 220 mg/dL LDL in adults
  - Normal level triglycerides

Severe FH patients are at a Very High Cardio Vascular Risk

A rare and life-threatening condition

- **Homozygous FH patients**
  - High mortality rate
  - CV events occurring at a very early age (as early as 18 months, with death as early as 3 years if left untreated)\(^1,3\)
  - Average life expectancy of only 23 to 25 years.

- **Severe Heterozygous FH patients**
  - Develop cardiovascular disease by age 30
  - CV events by the third or fourth decade of life\(^3\)
  - Significant risk of multiple cardiac events and premature death

Apolipoprotein B (APOB or ApoB) is the primary apolipoprotein of low-density lipoproteins (LDL or "bad cholesterol"), which is responsible for carrying cholesterol to tissues.

- The APOB on the LDL particle acts as a ligand for LDL receptors in various cells throughout the body.
- Absolutely required for LDL and VLDL formation.
- Blocking ApoB production blocks VLDL and LDL production.
- Through a mechanism that is not fully understood, high levels of APOB can lead to plaques that cause vascular disease (atherosclerosis), leading to heart disease.
Antisense Oligonucleotide (ASO) Drug

DNA encoding ApoB → mRNA for ApoB production → ASO drug

Translation

ApoB

Lipid Production

VLDL

LDL

Improved CV Health
Phase 3 Study: Immunogenicity Evaluation

- Patients were considered eligible for evaluation if they had a baseline and at least one post-baseline serum sample.
- Serum evaluated using anti-drug antibody (ADA) specific immunoassays:
  - Testing Scheme: ELISA screen/confirmatory IP assay/ELISA titer
- Time points:
  - Pre-treatment, end of study (6 mo)
  - Open Label Extension (OLE): End of observation period (6 mo) or OLE dosing (6 mo, additional time points)
Immunogenicity Evaluation: ADA ELISA

Protein A/G-HRP

Antibody response

Biotin-Drug

Streptavidin

Key to use buffers compatible with nucleic acid analysis

1 Hr
Capture

1 Hr
Block

2 Hr
Sample

1 Hr
Conjugate

30 Min
Substrate

READ

Confirmatory: SCIP Assay
Findings: Incidence of ADA

- Antibody positive samples were apparent during 6 months or more of dosing

- Incidence in Phase 3 and OLE studies
  - Approx. 37.5% positive binding to drug in immunogenicity assays in 6-month Phase 3 studies
  - Incidence increased in OLE (72%; averaged 18-month of study treatment)
  - Detected more ADA during drug wash-out period
  - Placebo: all patients were negative for antibodies
Three Representative Patient Antibody Response Profiles
Immunogenicity
ADA Effect on Efficacy, Safety and PK

- Efficacy in antibody positive patients similar to those who were negative
  - Mean % change in pharmacodynamics biomarkers from baseline – were not statistically different

- Safety in antibody positive and negative patients relatively similar
  - Including Adverse Events or clinical biomarkers
  - Exception: greater incidence Flu-Like Symptoms in antibody positive patients

- Apparent effect on trough drug PK levels – higher in antibody positive samples
Anti-dsDNA Assay

- Phadia ImmunoCAP100 EliA dsDNA kit
  - Fully-automated quantitative ELISA (human IgG std curve)
  - IVD approved

- Capture
  - Circular, double-stranded plasmid DNA antigen

- Detection
  - B-galactosidase conjugated mouse anti-human IgG

- Results
  - Negative <10 IU/mL
  - Equivocal 10-15 IU/mL
  - Positive >15 IU/mL
Cases: High ADA titers and Positive for anti-dsDNA
Summary

- Patients treated with Drug X who develop ADA may develop anti-dsDNA antibodies.
  - No anti-dsDNA antibodies detected in ADA negative patients or samples
  - No anti-dsDNA antibodies detected at baseline in any of the patients studied
  - Patients with higher ADA titers appear to have a greater risk of developing anti-dsDNA antibodies.

- Although anti-dsDNA antibodies are detected in the isolated cases, a full clinical assessment does not support an autoimmune diagnosis for any of Drug X treated patients at this time.
Conclusions

- ADA can develop to oligonucleotide therapeutics

- Immunogenicity bioanalysis paradigm developed for other classes of biotherapeutics should be followed

- ADA detection may be influenced by assay methodology, sample handling and timing of sample collection. ADA sample should be aligned with PK

- Immunogenicity risk assessment should be considered
Acknowledgements

Thank you