Immunogenicity of Biopharmaceuticals:

Potential Impact of Protein Characteristics

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Eli Lilly and Company, Indianapolis, IN USA

2010 Workshop on Protein Aggregation and Immunogenicity – Breckenridge, CO

Answers That Matter.
Immunogenicity of Biopharmaceuticals

Topics

• Immune response
• Drug characteristics
• Immunogenicity
• Risk-based assessment
• Immunogenicity in drug development
• Case studies demonstrating impact of immunogenicity
Immune Response

(a) Antigen
   Co-stimulatory signal
   APC (dendritic cell) → T_H cell
   → T_H activation
   → Effector + Memory T_H cells
   → IL-2
   → IL-2
   → IL-2

(b) Antigen
   Cytokines
   B cell → T_H cell
   → Memory B cell
   → Plasma cell
   → Memory T_C cell
   → CTL
   → Killing
   → Lysis

(c) T_C cell
   Altered self cell
   → IL-2
   → IL-2

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B cell Response

(b)

Antigen

Ag Presenting cell

Protein antigen

II. Epitope Capture

Antigen denaturation and binding to MHC-II

MHC-II-protein complex

Lysosomal proteases acting on MHC-II-bound antigens

Protection of bound peptide

B cell

Cytokines

TH cell

Memory B cell

Plasma cell

MHC-II-protein complex

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What triggers the immune response?

A general property of the immune system is antibody preference for conformational determinants, instead of linear epitopes.

The system detects pathogens via a set of receptors that recognize pathogen-associated molecular patterns (PAMPs).

A self-antigen (or mimetic) presented in a conformation that is “recognized” induces the immune response (T cell independent).

Or a self-antigen (or mimetic) presented with a companion “danger” signal (DAMPS: e.g. endotoxins, denatured proteins, host cell protein) may also induce an immune response (T-cell dependent).

Natural antigen with PAMP

**B-epitope**

- B-cell epitopes are peptides (usually conformational) or other biomolecules that bind specifically to antibodies.

- T-cell epitopes are short peptide sequences (usually linear) that elicit the cellular immune response of activated T-cell clones.

Biomolecule

Aggregated Biomolecule
What is Immunogenicity?

In the context of an immune response to an administered protein therapeutic, “Immunogenicity” refers to the production of host antibodies reactive with the biotherapeutic.
Factors Affecting the Immunogenicity of a Biotherapeutic Drug

**Product Specific**

- **Structural**
  - Size (m.w.)
  - Species-specific epitopes
  - Aggregation
  - Oxidation
  - Deamidation & Degradation
  - Glycosylation

- **Impurities**
  - Host Cell Protein
  - Endotoxin,
  - Protein A

- **Formulation**

- **Storage Conditions**
  - Temperature
  - Container

**Study Specific**

- **Host**
  - Health; immune status,
  - Genetic background
  - Homology
  - Co-meds

- **Dose**

- **Route**

- **Frequency**
What We Know

• Most biopharmaceuticals will induce antibodies
  ▪ Reaction to neo-antigens
  ▪ Breakdown of immune tolerance

• There are many factors that influence immunogenic responses

• It is a safety concern (risk-based scale)

• There are regulatory expectations to assess it

• The assessment & interpretation bar will continue to be raised…

  “The more we learn the less we know!”
Definitions

Immunogenicity
The ability of a substance (antigen) to induce an immune response

Anti-drug antibody (ADA) or anti-therapeutic antibody (ATA)
An immunoglobulin (Ig) with specific binding properties to a drug

Neutralizing anti-drug antibody (nADA or Nab, or nATA?)
An immunoglobulin (Ig) with specific binding properties to a drug that result in diminished or negated drug activity.
“Risk-Based Assessment”

Probability of inducing nADA

+ 

If nADA are induced,

Impact of potential adverse effects
## Risk-Based Assessment: How likely is a nADA response?

### Drug Characteristics

<table>
<thead>
<tr>
<th>How human is the drug?</th>
<th>Human</th>
<th>Humanized</th>
<th>Chimeric</th>
<th>Mouse</th>
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<tbody>
<tr>
<td>Homology to endogenous</td>
<td>Homologous</td>
<td>Partial</td>
<td>Fusion</td>
<td></td>
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<tr>
<td>Dosing Frequency</td>
<td>Single</td>
<td>Acute</td>
<td>Chronic</td>
<td>Intermittent</td>
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<td>Dose Concentration</td>
<td>Very High</td>
<td>Low - Average</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Route of Administration</td>
<td>Oral</td>
<td>i.v.</td>
<td>i.p.</td>
<td>s.c.</td>
</tr>
<tr>
<td>Clearance (t₁/₂)</td>
<td>Fast</td>
<td>Slow</td>
<td></td>
<td></td>
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<tr>
<td>Aggregates present?</td>
<td>No</td>
<td>Yes</td>
<td></td>
<td></td>
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</table>

### Patient Characteristics

<table>
<thead>
<tr>
<th>Patient Immune Status</th>
<th>Suppressed</th>
<th>Normal</th>
<th>Activated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune-modulator?</td>
<td>Immunosuppressant</td>
<td>Immunostimulant</td>
<td></td>
</tr>
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</table>

### Probability =

| Low | Unknown | High |

*Modified from Info Provided by Eric Wakshull*
Risk-Based Assessment:
How Serious could nADA response be?

<table>
<thead>
<tr>
<th>Question</th>
<th>No (or replacement)</th>
<th>Redundant</th>
<th>Unique</th>
<th>Tolerizable</th>
<th>Manageable</th>
<th>Fatal</th>
<th>Yes – no MTD</th>
<th>No – Low MTD</th>
<th>Tolerizable</th>
<th>Manageable</th>
<th>Fatal</th>
<th>Life-Threatening</th>
<th>Not Life-Threatening</th>
<th>Intended Disease</th>
<th>Life-Threatening</th>
<th>Not Life-Threatening</th>
<th>Tolerizable</th>
<th>Manageable</th>
<th>Fatal</th>
<th>Life-Threatening</th>
<th>Not Life-Threatening</th>
<th>Treatment Options</th>
<th>No options</th>
<th>Other options available</th>
<th>Potential cross-linking?</th>
<th>No</th>
<th>Yes (Reverses Antagonist/Blocking to Activating)</th>
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<tbody>
<tr>
<td>Endogenous homolog?</td>
<td></td>
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<td>Redundant/Unique Biology?</td>
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<td>Impact of autoimmune KO?</td>
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<td>Potential cross-linking?</td>
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<td>Impact</td>
<td>Less Serious</td>
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<td>More Serious</td>
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</tbody>
</table>

Always “Guarded”

Modified from Info Provided by Eric Wakshull
More Definitions

by A.G. Renwick, Principles and Methods of Toxicology, 3rd Ed. 1994

Pharmacokinetics (PK)

The movement of drugs within the body: adsorption, distribution, metabolism, excretion (ADME).

Pharmacodynamics (PD)

The pharmacological actions of the drug within the body.

Toxicokinetics (TK)

The application of PK principles to animal toxicity studies to provide information on exposure to the parent compound and metabolites, accumulation during chronic exposure, etc…
Potential Consequences of ADA

- None
  - ADA are present but have no consequence

- Alteration of PK/PD
  - Decrease drug exposure due to ADA
    - Increase rate of clearance
    - Interference in PK assay (apparent decrease in PK)
  - Increase drug exposure due to ADA (act as “carriers”)

- Adverse Effects
  - Hypersensitivity, IgE
  - Immune complex formation
  - Neutralization of endogenous protein – autoimmune
Characteristics of ADA and impact:

*combinations of these characteristics may exist*

**Binding**  
ADA bound to drug. No apparent effect on drug PK or PD.

**Sustaining**  
ADA:drug complex prolongs the circulating half-life of the drug  ⇒ Increased drug exposure.

**Clearing**  

**Neutralizing**  
ADA:drug complex prevents target binding activity of drug.  ⇒ Decreased efficacy  
⇒ Autoimmune response
Neutralizing ADA have the potential to:

- Prevent the movement of drugs within the body (PK/TK)
- Block the pharmacological actions of the drug within the body (PD)

Worst Case:

- Induce an adverse event
- Invalidate a Tox Study
  - animals not exposed to active drug
Potential Impact of ADA on Nonclinical Safety “Tox” Study

In the presence of ADA, a Tox study is “validated” by performance of an assessment of exposure to active drug or demonstration of bioactivity.

• confirm that the drug remained active at expected exposure rates during the safety evaluation in the test subjects.
  
  – PK profile (drug exposure)
  
  – PD profile (blood pressure, calcium level, other biomarkers)
  
  – Adverse events - “Toxicodynamics”
  
  – Immunogenicity characterization (Neutralizing Antibody Assay)
Example:

**PK Profile**

- **tAb1**

- **Dose**: q7d x 2 on days 0 and 7, 25 mg/kg each

- **Expected TK profile**

- **“Altered” TK Profile**
Alterations in PK

Possible causes for decreased drug exposure?

- **Drug Characteristics** - ADME
  - Adsorption
  - Distribution
  - Metabolism
  - Excretion
  
  - Usually these parameters or more clearly applied to small molecules, however, they should be considered for biomolecules as well.

- **Immunogenicity**
Example:

tAb1

PK Profile

q7d x 2 on days 0 and 7, 25 mg/kg each

"Altered" TK Profile

Expected TK profile

Concentration (µg/mL)

hours

Dose

Dose

ADA+

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tAb Alterations in PK

Possible Causes?

• **Binding ADA** - PK assay interference (drug is there, but cannot be measured by PK immunoassay due to ADA interference)

• **Clearing ADA** have removed drug from circulation

• **Neutralizing ADA** - PK assay interference (drug is there, but cannot be measured by target-binding format PK assay)

• Pharmacological activity of the drug – Biofeedback mechanism causes influx of drug target in serum which competes with PK immunoassay (*assay interference; not ADA-related*)
ADA interference in PK **Antigen-Capture** Immunoassay

ADA prevent Drug (therapeutic Ab, “tAb”) from binding to Target on ELISA plate

It “appears” that drug exposure is reduced
To evaluate “active drug” exposure….

- confirm that the drug remained active at expected exposure rates during the safety evaluation in the test subject.
  - PK profile (drug exposure)
  - ADA screen results
  - PD profile (blood pressure, calcium level, other biomarkers)
  - Adverse events - “Toxicodynamics”
  - Immunogenicity characterization (Neutralizing Antibody Assay)
Case Studies
Case Study #1:
Tox Nonclinical Safety Study

Drug: “Protein X” variant – a recombinant modified endogenous protein

Rhesus Monkey (>95% homology to human Protein X)

Dosing: S.C.; Daily for 32 days

Samples collected for:
• PK
• ADA
• Standard Tox Study parameters & tissues
# Case Study #1:

## Test Article Characterization

<table>
<thead>
<tr>
<th>Assay</th>
<th>Analytical Property</th>
<th>Result</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein Content (UV)</td>
<td>rProtein X</td>
<td>26.0</td>
<td>mg/mL</td>
</tr>
<tr>
<td>Purity (RP-HPLC)</td>
<td>rProtein X</td>
<td>96.0</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>Total Impurities</td>
<td>4.0</td>
<td>%</td>
</tr>
<tr>
<td>Soluble Aggregates (SEC)</td>
<td>Monomer</td>
<td>100.0</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>Total Polymer</td>
<td>0.03</td>
<td>%</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>rProtein X</td>
<td>100.0</td>
<td>%</td>
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<tr>
<td>Bioassay</td>
<td>Bioactivity</td>
<td>Active</td>
<td>Active/Inactive</td>
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<td>Host Cell Protein</td>
<td>Host Cell Protein: Yeast</td>
<td>35</td>
<td>ppm</td>
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<td>DNA (Threshold)</td>
<td>DNA</td>
<td>0.91</td>
<td>ppb</td>
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<td>Endotoxin</td>
<td>Bacterial Endotoxins</td>
<td>0.02</td>
<td>EU/mg</td>
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<td>cIEF</td>
<td>pI</td>
<td>5.08</td>
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<td>LC/MS</td>
<td>MW</td>
<td>&gt;10k</td>
<td>Da</td>
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<td>N-Terminal Sequencing</td>
<td>N-Terminal Sequence</td>
<td>Matches expected sequence</td>
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<tr>
<td>Amino Acid Analysis</td>
<td>Amino Acid Composition</td>
<td>Matches expected amino acid composition</td>
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</table>
rProtein X: PK and ADA Screen Results

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rProtein X  Study Interpretation

• PK assay confirms drug exposure
• ADA screen positive screen
• No Adverse Events – no Toxicity

= “Clean” study ?

or….

Did ADA neutralize the activity of the drug?
To evaluate “active drug” exposure….

- confirm that the drug remained active at expected exposure rates during the safety evaluation in the test subject.
  - PK profile (drug exposure)
  - ADA screen results
  - PD profile (e.g. blood pressure, calcium level, other biomarkers)
  - Adverse events - “Toxicodynamics”

? Immunogenicity characterization (Neutralizing Antibody Assay)
rProtein X  Considerations…

• PD marker not available for this Study
  - At the time of this study, PD marker only observed in disease-state animals
  - Not feasible to have disease-state Tox animals

• Format of PK assay is not target-binding. ADA can bind to Drug, not be cleared, and can still be measured…Can’t use “Ligand-binding assay”
rProtein X: PK Assay

Antibody Capture Assay – not sensitive to ADA
rProtien X: Risk Assessment

• The Drug is a modified human endogenous protein
• Limited knowledge of activity/pathway
• Conclusion: “High” risk

Decision: Test for neutralizing capability of the ADA

– Serum-tolerant cell-based bioassay was developed
  • 5 months to develop & validate
– Results = “non-neutralizing”
√ Confirmed in subsequent longer studies with PD
Case Study #2:
Tox Nonclinical Safety Study Results

- PK by Ag-Capture appears to have a faster clearance rate on Day 36.
  - Sensitive to ADA interference?
  - Reduced Exposure?

- Total Human IgG has similar profile to Day1.
  - Drug is in circulation

- No adverse events were observed

- Unexpected PK with ADA+ suggests potential for neutralization of the activity of the drug.
## Case Study #2:
### Test Article Characterization

<table>
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<th>Assay</th>
<th>Analytical Property</th>
<th>Result</th>
<th>Units</th>
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</thead>
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<td>UV</td>
<td>Protein Content</td>
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<td>Monomer</td>
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<td>%</td>
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<td>Polymer</td>
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<td>%</td>
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<td>Soluble Aggregates (SEC)</td>
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**Case Study #2:**

**ADA Data** (6 week wash-out)

<table>
<thead>
<tr>
<th>Group</th>
<th>Study Animal Incidence / Total Animals in Group</th>
<th>Titer Range of Positives</th>
<th>Potential for False Low or Negative due to Drug Interference</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>0 / 12</td>
<td>none</td>
<td>none</td>
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<tr>
<td>Low (3 mpk)</td>
<td>1 / 6</td>
<td>125</td>
<td>Low</td>
</tr>
<tr>
<td>Mid (30 mpk)</td>
<td>4 / 6</td>
<td>5 to 625</td>
<td>High</td>
</tr>
<tr>
<td>High (150 mpk)</td>
<td>7 / 12</td>
<td>5 to 625</td>
<td>High</td>
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Case Study #2: PD Biomarker Considerations

Target Protein elevates in dose-responsive manner within hours following Drug administration.

Target Protein can be used as a PD Biomarker

Caution: Both Drug and Target Protein have potential interference with the ADA assay
Impact of ADA on PK (Ag capture)
Comparison of Day 1 and Day 36

Drug    PK    AUC   (Linear scale)
Animal #
Dose (mg/kg/week)

ADA Titer Drug AUC Day 1 (ug*hr/mL) Drug AUC Day 36 (ug*hr/mL)

Impact of ADA on PK (Ag capture)
Comparison of Day 1 and Day 36

6/12
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Impact of ADA on PD Biomarker
Comparison of Day 1 and Day 36

- ADA Titer
- PD Biomarker Day 1 AUC (ng*hr/ml)
- PD Biomarker Day 36 AUC (ng*hr/mL)

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Case Study #2

Study Conclusions

• It appears that animals with the highest ADA titers are associated with lower PK and PD marker AUCs.

• …

• Although high ADA titers appear to impact pharmacodynamics, PD marker AUCs in the 150 mg/kg dose group remained above those of the control group, which suggests continued active Drug pharmacology.
Summary

Many biopharmaceutical product characteristics, including aggregation, are associated with immunogenicity risk.

The potential to increase the incidence/titer of ADA may impact a nonclinical or clinical study:

• Induce Adverse Event
• Decrease drug efficacy
• Confound/invalidate the Safety Assessment
• May add additional time and cost to drug development for ADA characterization or product manufacturing changes.
Acknowledgements

Carolyn Halstead  Immunogenicity
Elizabeth Galbreath  Pathology
David Waters  Toxicokinetics
Jennifer Martin  Toxicokinetics