Immunogenicity Assessment
- Challenges and Strategies

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Introduction

Immunogenicity Testing (ADA and NAb)

Challenges and Strategies
- Matrix interference
- Drug Tolerance
- Target Interference

Summary
Biological Therapeutics

- Peptides
- Proteins (recombinant)
  - Pegylated Proteins
  - Fusion Proteins
  - Chimeric Proteins
- Oligonucleotides
- Antibody Therapeutics
  - Monoclonal antibodies
  - Chimeric antibodies
  - ScFV
  - Bispecific antibodies
  - Antibody Drug Conjugates (ADCs)
- Biosimilars and Biobetters
What is Immunogenicity?

“The Ability of a Substance (e.g. Antigen or Vaccine) to Elicit an Immune Response”

- Innate Immunity
- CDC
- Hypersensitivity
- Anti-drug Antibodies
- Cytokine Storm
- ADCC
- Cell Mediated Cytotoxicity
- T Cell Activation
# Immunogenicity Drivers

<table>
<thead>
<tr>
<th>Intrinsic</th>
<th>Extrinsic</th>
</tr>
</thead>
<tbody>
<tr>
<td>❖ Self/non-self <em>(antigenicity: T and B cell epitopes in a protein)</em></td>
<td>❖ Mode of Administration <em>(oral, im, sc, iv)</em></td>
</tr>
<tr>
<td>❖ Monomeric/multimeric</td>
<td>❖ Dose &amp; Frequency of administration</td>
</tr>
<tr>
<td>❖ Fusion Proteins</td>
<td>❖ Excipients and Formulants <em>(adjuvant effect)</em></td>
</tr>
<tr>
<td>❖ Glycosylation</td>
<td>❖ Aggregates</td>
</tr>
<tr>
<td>❖ Indication <em>(User’s immune system status eg: Rituxan strongly immunogenic in RA patients)</em></td>
<td>❖ Drug Effector function <em>(CDC, opsonization-immune stimulation; ADCC-killing/ modulation of immune cell function)</em></td>
</tr>
<tr>
<td>❖ Genetics, age, gender</td>
<td>❖ Degradation Products</td>
</tr>
<tr>
<td></td>
<td>❖ Production Contaminants <em>(leachates and extractables)</em></td>
</tr>
</tbody>
</table>
## Consequences of Clinical Immunogenicity

<table>
<thead>
<tr>
<th>Clinical Concern</th>
<th>Clinical Outcome</th>
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| **Safety**      | - Cross-reactivity with an endogenous counterpart, may neutralize activity with unique function causing deficiency syndrome  
- Acute consequences (infusion reactions, allergic reactions, anaphylactic reactions)  
- Non-acute consequences (delayed type hypersensitivity/ immune complexes) |
| **Efficacy**    | - Enhancing or decreasing efficacy by extending or decreasing half life.  
- Enhancing or decreasing efficacy by changing bio-distribution. |
| **Pharmacokinetics** | - Antibody production may dictate changes in dosing levels due to PK changes |
| **None**        | - Despite generation of antibodies, no discernible impact. |

Ref: Susan L. Krishner (CDER/FDA) presentation on Assessment of Immunogenicity of Biological Therapeutics 2009
# Types of Anti-Drug Antibody (ADA)

<table>
<thead>
<tr>
<th>Characteristics of ADA and its Impact</th>
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</table>
| **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:** ADA:drug complex cleared from circulation
  => Decreased drug exposure |
| **Neutralizing:** ADA:drug complex prevents target binding activity of drug
  => Decreased efficacy
  => Autoimmune response |
Real Life Examples

Immune responses to therapeutic proteins resulted in devastating consequences for healthy volunteers and patients

<table>
<thead>
<tr>
<th>Loss of Efficacy</th>
<th>Enhancement of Efficacy</th>
<th>Neutralization of Endogenous Protein</th>
<th>General Immune Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptokinase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcitonin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor VIII</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interferon alpha 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interferon beta</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM-CSF/IL3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth Hormone</td>
<td></td>
<td>rDNA Human MGDF (Megakaryocyte-derived growth factor)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Erythropoietin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Allergy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anaphylaxis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serum sickness, etc</td>
</tr>
</tbody>
</table>
Drug candidates need to be evaluated for potential adverse events
- Immunogenicity
- Allergenicity

Need to Develop Several Bioanalytical Assays
- Anti drug-specific antibodies (ADA)
- Anti-drug neutralizing antibody (NAb) assay – cell based functional assay
- In cases of severe adverse drug reactions (hypersensitivity)
  - Determine if SAEs are related to IgE-mediated responses
    - Serum drug-specific IgE (ELISA)
    - Basophil activation test (BAT using whole blood)
Immunogenicity – ADA Assay Formats

- **Direct method**
  - Colorimetric
  - Chemiluminescence
- **Acid Dissociation**
  - Master Mix (ECL-based)
  - Solid Phase Extraction (SPEAD)
  - Affinity Capture Elution (ACE)

- **Bridging method**
  - ELISA
  - Electrochemiluminescence (ECL-based)

- Pre-treatment with target coupled *nanobead / magnetic bead* to reduce drug interference
Multi-tiered Approach for Immunogenicity

Anti-Drug Antibody Assay (ADA)
- **Screening assay** for rapid identification of positive samples
- **Confirmatory assay** to confirm results of screening assay
- **Titer assay** to measure relative levels of ADA

Neutralizing Antibody Assay (NAb)
- **Functional assay** for assessment of neutralizing activity
Challenges in Immunogenicity Testing

- Matrix Interference
- Drug Interference
- Target Interference
Drug Interference

- Many of the therapeutic drugs, particularly an antibody therapeutic, have a long half-life (typically 10-20 days) and often administered chronically at high dose levels without a drug wash-out period.

- Immunogenicity assessments to such therapeutics pose unique challenges in clinical trials especially when significant drug interference is encountered.

- Free drug can complex with anti-drug antibodies directly interferes with the assay.

- Tolerance of the ADA assay for presence of drug to be determined.
Drug Interference: FDA’s Position

2009 Draft Guidance for Industry

- Drug interference

“Of greatest concern is the presence of product in matrix…..”

- FDA recommends the applicant address such possibilities early (preclinical and Phase I or early phase 2)

- FDA provides no official advice on how to address this issue other than sampling after a washout period (e.g., five drug half-lives)
Drug Interference: EMEA’s Position

2007 EMEA Guidance on Immunogenicity Assessment

- Drug interference

“Residual biological product present in patients’ blood can complex with induced antibody and hence reduce the amount of antibody detectable by assays”

- EMEA does provide suggestions for dealing with this issue

“...it may be circumvented/resolved by using a number of approaches e.g. by dissociating the immune complexes with acid, removing excess biologicals by solid-phase adsorption and or using sufficient sample dilution”
Drug interference can obscure the ability to detect ADA positive samples

Dilute antibody samples with varying amounts of drug to assess how much drug is required to reduce response below the cut point
Approaches to Improve Drug Tolerance

- Acid Dissociation
- Incubation Times
- Sample Dilution

Advantages:
- Improved ADA detection
- Shorter washout periods
Concerns about Acid Dissociation

- Acid dissociation procedure may result in loss of signal due to specific loss of a sub-class of ADA.
- Low pH may result in irreversible denaturation/loss of some ADA.
- Risks associated with Acid treatment of Samples for ADA detection:
  - Under estimation of ADA levels?
  - Is there an impact on Data interpretation?
Optimization of Acid Dissociation Step

- Optimal conditions varies from one therapeutic to another
- Depends on the characteristics of the therapeutic and available reagents
- Use of DOE is recommended for optimization of sample acidification/neutralization conditions
  - Optimal pH and concentration of acetic acid
  - Duration of acidification
With and Without Acid Dissociation

Case Study A
Use of Acid Pretreatment in ADA Assay

- ADA method revalidated to improve drug tolerance
- Utilize the acid pretreatment step followed by neutralization in presence of optimal concentration of labeled Drug-X
- Optimal MSD Plate – MSD® Streptavidin Gold plate

<table>
<thead>
<tr>
<th>Validation Parameter</th>
<th>Old ECL- based Validated Method</th>
<th>New ECL-based with Acid Dissociation Validated Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Sensitivity in neat serum</td>
<td>10.0 ng/mL</td>
<td>4.50 ng/mL</td>
</tr>
<tr>
<td>(Interpolated value)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug Tolerance (interpolated value)</td>
<td>30.0 µg/mL</td>
<td>150 µg/mL</td>
</tr>
</tbody>
</table>

Case Study A
Drug-Y (example of an ADC compound)

- MSD® High Bind Avidin plates – optimal for Drug-Y
- Utilizes the acid treatment step followed by neutralization in presence of optimal concentration of labeled drug (Biot-Drug-Y and Sulfo-Drug-Y)

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Assay Sensitivity (ng/mL)</th>
<th>Drug Tolerance (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Serum</td>
<td>2.00 ng/mL</td>
<td>60.0 µg/mL</td>
</tr>
<tr>
<td>Cynomolgus Monkey Serum</td>
<td>3.00 ng/mL</td>
<td>&gt;100 µg/mL</td>
</tr>
</tbody>
</table>
Drug Z: Comparison of Drug Tolerance

Significant increase in Drug Tolerance especially samples containing low levels of ADA

Case Study C
Target Interference

- Target interference is also a big concern in bridging immunoassays, especially when target is a soluble receptor.
- Target interference can also be intensified by AD which then release target from target-drug complex.
- Several options to reduce potential target interference:
  - Sample pretreatment with magnetic beads/nanoparticles coated with……
    - Use of anti-target antibody
    - Use of competing ligand for the target
Immunogenicity (Neutralizing Ab Assay)

- **Cell Based Assays**
  - Cell Proliferation
  - Apoptosis (Caspase)
  - cAMP Agonist Assay
  - Cytokine release
  - Reporter gene Assay

- **Non-Cell Based Assays**

- **Competitive Ligand Binding Assays**
Detect NAb Using Proliferation Assay

- **Cell line is dependent on drug for proliferation**
- **Add drug and sample**
- **Add Detection Reagent**
- **Read**

**NAb Negative Sample**
- High Signal

**NAb Positive Sample**
- Low Signal
Serum Matrix Interference

- Growth factors
- Serum proteins
- Complement
- Cytokines
- Chemokines
- Endogenous protein (if any)

- Serum Dilution
- Melon gel IgG Purification
- Heat Inactivation
Sample Pretreatment: Heat Inactivation at 56°C for 30 min
Basophil Activation Test (BAT) Method to Assess Drug-induced IgE mediated Hypersensitivity
FACS profile: Stained Blood Leukocytes

Ref: Buhlmann’s Flow2 CAST Instruction Booklet inside the kit (purchased through Alpco Diagnostics)
Schematic Diagram: BAT Method

Basophil Activation Test (BAT) Method
Basophils are identified on the basis of CCR3 on their surface (further discriminated from eosinophil population by size and granularity).

Resting basophils do not express CD63 (anchored in the basophilic granule) and weakly express CD203c on their cell surface.

The cross-linking of two FCεRI (induced by a drug/allergen) initiates a cascade of signaling events that leads to histamine release and consequently, CD63 expression and the up-regulation of CD203c on activated basophils.

The increase in CD63 and/or CD203c expression after drug/allergen challenge reflects the basophil activation/de-granulation in response to a drug/allergen.
Schematic Diagram: BAT Method

Basophil Activation Test (BAT) Method
FACS profile: Stained Blood Leukocytes

- Basophils
- Eosinophils
- Monocytes
- Neutrophils
- Lymphocytes

Ref: Buhlmann’s Flow2 CAST Instruction Booklet inside the kit (purchased through Alpco Diagnostics)
Flow-Cytometric BAT method based on the detection of drug/allergen-induced CD63 expression.

Key parameters
- Blood processing time
- Blood sample volume
- Incubation/stimulation time
- Selection of positive and negative controls

Drug-specific Chimeric mHu-IgE antibody used as a positive control

Demonstrated the binding of Chimeric mHu-IgE to the FCɛRI on human basophils

Binding induced sensitization of basophils thus resulted in up-regulation of CD63 expression
Use of Chimeric IgE (drug-specific) as PC

=> Significant expression of CD63 on pre-sensitized basophils upon stimulation with Drug
Summay

- **Heat inactivation** can be used as one of the approach to minimize matrix interference in ADA and NAb assays.

- **Acid dissociation** is still a very popular technique to improve drug tolerance.

- Thorough optimization of acid pretreatment step is critical to minimize irreversible denaturation/loss of some ADA.

- Multiple acid dissociation steps can be implemented in reducing both soluble target and drug interference in an ADA assay.

- Acid dissociation approach has been successfully applied to develop and validate improved ADA assays for multiple biologics including therapeutic antibodies, proteins, antibody drug conjugates.

- **BAT assay** can be used to test drug induced IgE mediated hypersensitivity.
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