Technical and Logistic Challenges in the Detection of Immunogenicity

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Immunogenicity & Clinical Relevance

What attributes of ADA immune response have the potential to be clinically relevant?

- Preexisting ADA
- Treatment-induced ADA
- Treatment-boosted ADA
- ADA level/titer
- Antibody isotype
- ADA cross-reactivity with an endogenous component and related biological drug
- ADA duration (persistence)
- Time to ADA onset
- Neutralizing ADA
- Drug-clearing ADA response
- Drug-sustaining ADA response
- For multi domain molecules, the domain specificity of ADA

What types of immunogenicity-related adverse clinical consequences are possible?

**Acute adverse events:**
- Type-I hypersensitivity
- Injection-site reaction or infusion reaction

**Non-acute adverse events:**
- Type-III hypersensitivity
- Worsening of disease
- Increased drug toxicity
- Partial response (attenuated efficacy)
- Primary loss of response
- Secondary loss of response

Shankar et al, Nature Biotechnol 2015, 33, 334-6

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Anti-Drug Antibody (ADA) Formation

Possible Effect on PK and PD

- Neutralizing Antibodies
  - Protein-ADA Complex
    - CL ↓
    - CL ↑
  - Immunogenic Response
  - Non-Neutralizing Antibodies
  - Exposure ↑ Activity ↓
  - Sustaining ADA

- Clearing ADA
  - Exposure ↓ Activity ↓
  - Protein-ADA Complex
    - CL ↑
    - CL ↓
  - Sustaining ADA
  - Exposure ↑ Activity ↑
ADA Assessment

Multi-Tiered Approach

- Determining the incidence, magnitude and impact of an immune response

Test specimens

- Including baseline samples for pre-existing ADA

Screening Assay

- IgG IgM (IgA IgE)

Positive specimens

Confirmatory Assay

- Confirmed positive specimens

Negative specimens

Tier 1 - Screening

- Drug concentration > drug tolerance

Inconclusive

Screening Assay

Tier 2 - Confirmation

- Test specimens Including baseline samples for pre-existing ADA

Confirmed positive specimens

Characterization Assays

- Correlation with PK, PD and biomarker measures

Tier 3 - Characterization

- Isotyping Domain/epitope mapping Cross-reactivity

Neutralizing Assay

Titer Assay

Inconclusive

Drug concentration > drug tolerance

Correlation with PK, PD and biomarker measures
Immunogenicity Assessment

FDA Guidance

Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

January 2019
Pharmaceutical Quality/CMC
# Immunogenicity Incidence Rates

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Technology</th>
<th>Antigen</th>
<th>Comedication</th>
<th>Immunogenicity incidence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKT³</td>
<td>Mu</td>
<td>Yes</td>
<td>Yes</td>
<td>80%</td>
<td>Hooks et al. [1991]</td>
</tr>
<tr>
<td>Zevalin™</td>
<td>MuRC</td>
<td>Yes</td>
<td>Yes</td>
<td>3.8%</td>
<td>PI</td>
</tr>
<tr>
<td>Bexxar®</td>
<td>MuRC</td>
<td>Yes</td>
<td>Yes</td>
<td>10–70%</td>
<td>PI</td>
</tr>
<tr>
<td>ReoPro®</td>
<td>Ch</td>
<td>No</td>
<td>No</td>
<td>5.8% (1 dose)</td>
<td>PI, Techeng et al. [2001]</td>
</tr>
<tr>
<td>Rituxan®</td>
<td>Ch</td>
<td>Yes</td>
<td>No</td>
<td>1.1%</td>
<td>PI</td>
</tr>
<tr>
<td>Simulect®</td>
<td>Ch</td>
<td>Yes</td>
<td>Yes</td>
<td>1.2–3.5%</td>
<td>PI</td>
</tr>
<tr>
<td>Remicade®</td>
<td>Ch</td>
<td>Yes</td>
<td>Yes</td>
<td>10–61%</td>
<td>PI, Baert et al. [2003]</td>
</tr>
<tr>
<td>Erbitux™</td>
<td>Ch</td>
<td>No</td>
<td>Yes</td>
<td>5%¹</td>
<td>PI</td>
</tr>
<tr>
<td>Zenapax®</td>
<td>Hz</td>
<td>Yes</td>
<td>Yes</td>
<td>8.4%</td>
<td>PI</td>
</tr>
<tr>
<td>Synagis®</td>
<td>Hz</td>
<td>No</td>
<td>No</td>
<td>0.7–1.8%</td>
<td>PI</td>
</tr>
<tr>
<td>Herceptin®</td>
<td>Hz</td>
<td>No</td>
<td>Yes</td>
<td>0.1%</td>
<td>PI</td>
</tr>
<tr>
<td>Mylotarg™</td>
<td>HzTC</td>
<td>Yes</td>
<td>Yes</td>
<td>0% HAHA 2 pts. HATA</td>
<td>PI</td>
</tr>
<tr>
<td>Campath®</td>
<td>Hz</td>
<td>Yes</td>
<td>No</td>
<td>1.9% CLL patients</td>
<td>PI, Weinblatt et al. [1995]</td>
</tr>
<tr>
<td>Xolair®</td>
<td>Hz</td>
<td>No</td>
<td>No</td>
<td>&lt;0.1%</td>
<td>PI</td>
</tr>
<tr>
<td>Raptiva™</td>
<td>Hz</td>
<td>Yes</td>
<td>No</td>
<td>6.3%</td>
<td>PI</td>
</tr>
<tr>
<td>Avastin™</td>
<td>Hz</td>
<td>No</td>
<td>Yes</td>
<td>ND²</td>
<td>PI</td>
</tr>
<tr>
<td>Humira™</td>
<td>HuPD</td>
<td>Yes</td>
<td>Yes</td>
<td>1% with MTX 12 % monotherapy</td>
<td>PI</td>
</tr>
</tbody>
</table>

Data are from the product prescribing information (PI) or other references as indicated. Mu: murine; MuRC: murine radioconjugate; Ch: chimeric; Hz: humanized; HzTC: humanized toxin conjugate; HuPD: human phage-display derived. MTX: methotrexate. The immunosuppression columns indicate whether the antibody-antigen interaction is immunosuppressive and if immunosuppressive therapies are generally given concurrently with the antibody.

Roskos et al., Drug Develop Res 2004, 61, 108-20
Immunogenicity Assessment

Typical ADA Assay Formats

Sandwich format

\[
\text{Antibody} + \text{Drug} \xrightarrow{\text{Wash}} \text{Drug} + \text{Drug} \xrightarrow{\text{Wash}} \text{Drug} \xrightarrow{\text{Positive}}
\]

Bridging format

\[
\text{Antibody} + \text{Drug} + \text{Drug} \xrightarrow{\text{Wash}} \text{Drug} \xrightarrow{\text{Positive}}
\]
# Immunogenicity Assessment

## Major Differences between PK and ADA Assays

<table>
<thead>
<tr>
<th>Drug/Biomarker Assay</th>
<th>ADA Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCMS or Ligand binding assay</td>
<td>Ligand binding assay</td>
</tr>
<tr>
<td>Measured against identical, unique, known molecule</td>
<td>Measured against a ‘family’/mixture of unknown, species-different molecules with certain common properties</td>
</tr>
<tr>
<td>Positive controls/calibration standards available</td>
<td>No definitive positive controls available</td>
</tr>
<tr>
<td>Quantitative assessment</td>
<td>Only qualitative or semi-/quasi-quantitative assessment (titer)</td>
</tr>
<tr>
<td>Robust towards interferences</td>
<td>Specificity, sensitivity and tolerance against interfering substances are unique for each assay and different for each drug and on different assay platforms</td>
</tr>
</tbody>
</table>
### ADA Assay Challenges

**Lack of Suitable Positive Controls**

- No positive controls commercially available
- ‘Surrogate’ ADAs usually generated with a human therapeutic protein in animals (sheep, goat)
  - Most often polyclonal
  - Species difference with regard to affinity, epitope specificity
  - Different epitope binding as human protein is foreign for animals
- Later programs might use affinity purified patient positive controls
- Reagent continuity is challenging
- Semi-quantitative assessments
  - Even ‘titer assessments’ are only quasi-quantitative approaches
  - Quasi-units or titers (dilution steps)

⇒ Cross comparison across assays and platforms is INAPPROPRIATE
ADA Assay Challenges

Drug Tolerance - Acid Dissociation

(a) Without Acid dissociation

(Without Acid dissociation)

ADA is not detected. False negative result

(b) Acid dissociation

(With Acid dissociation)

Neutralize

ADA is detected. Accurate result

Gunn et al., Clin Exp Immunol 2016, 184, 137-146
ADA Assay Challenges

Soluble Target Interference

Gunn et al., Clin Exp Immunol 2016, 184, 137-146
Zhong et al., AAPS J 2017, 19, 1564-75

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Immune Complex Formation

TS1 and its monoclonal anti-idiotype, $\alpha$TS1

- The electron micrograph shows
  a. unreacted molecules
  b. chains of three
  c. rings of four
  d. a ring of six
  e. a ring of 10

Electron micrograph of TS1/$\alpha$TS1 immune complexes (0.1 mg/mL) 1:1 mixed, incubated for 20 min, and diluted 10-fold just prior to mounting and staining

Johansson et al., Cancer 2002, 94, 1306-13
Immunogenicity Interpretation

Complicating Factors in Clinical Assessment

- Heterogeneous response: Polyclonal and relatively unspecific
  - May be against one or multiple different epitopes
  - May vary greatly in affinity: High vs. low affinity
  - Antibody response = all antibodies generated in a patient in response to a drug
    - Clearing Ab vs. Sustaining Ab vs. Neutralizing Ab
  - One patient may form multiple different antibodies in response to a drug; different patients may have different responses
  - Effect of sustaining vs. clearing antibodies is largely determined by the formed ICs and the size of protein therapeutic

- Subjects/patients may have anti-Abs before first exposure
  - Sampling prior to first exposure is crucial
# Monoclonal Antibody Biosimilars

## Table 1 | Selected key products for which monoclonal antibody biosimilars are in development

<table>
<thead>
<tr>
<th>Product</th>
<th>Molecule</th>
<th>Number of biosimilars in development</th>
<th>Notable late-stage manufacturers</th>
<th>2014 sales (US$ billions)</th>
<th>Originator</th>
<th>US patent expiry*</th>
<th>EU patent expiry*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immunology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enbrel</td>
<td>Etanercept*</td>
<td>27</td>
<td>Merck/Samsung Bioepis, Coherus, Sandoz</td>
<td>8.5</td>
<td>Amgen/Pfizer</td>
<td>2028*</td>
<td>2015</td>
</tr>
<tr>
<td>Humira</td>
<td>Adalimumab</td>
<td>24</td>
<td>Amgen, Sandoz</td>
<td>12.5</td>
<td>AbbVie</td>
<td>2016</td>
<td>2018</td>
</tr>
<tr>
<td>Remicade</td>
<td>Infliximab</td>
<td>13</td>
<td>Celltrion, Hospira</td>
<td>9.2</td>
<td>Johnson &amp; Johnson/Merck</td>
<td>2018</td>
<td>2015</td>
</tr>
<tr>
<td><strong>Oncology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avastin</td>
<td>Bevacizumab</td>
<td>22</td>
<td>Amgen, Oncobiologics</td>
<td>7.0</td>
<td>Genentech/Roche</td>
<td>2019</td>
<td>2022</td>
</tr>
<tr>
<td>Herceptin</td>
<td>Trastuzumab</td>
<td>37</td>
<td>Actavis/Amgen/Synthon, Biocad, Biocon/Mylan</td>
<td>6.8</td>
<td>Genentech/Roche</td>
<td>2019</td>
<td>2014</td>
</tr>
<tr>
<td>Rituxan</td>
<td>Rituximab</td>
<td>44</td>
<td>Sandoz, Boehringer Ingelheim</td>
<td>8.7</td>
<td>Biogen/GeneTech/Roche</td>
<td>2018</td>
<td>2013</td>
</tr>
</tbody>
</table>

*The date given is based on the expected expiry of patents protecting the original molecule. †Etanercept is a fusion protein, composed of the tumour necrosis factor (TNF) receptor fused to the immunoglobulin G1 Fc domain. ‡The patent on Enbrel was originally set to expire in 2017, but Amgen received an additional 17 years of patent protection owing to a patent dispute; sources include company financial records, the Generics and Biosimilars Initiative (GABI), BioProcess International and BioPharm International. In the United States, the ‘molecule’ patents protecting the active ingredient etanercept have all expired aside from US8063182 and US8163522 members from priority CH331989 (1989-09-12) owned by Roche (exclusively licensed to Amgen), which are set to expire in 2028 and 2029, respectively.

Upda & Million, Nat Rev Drug Discov 2016, 15, 13-4
Example: Adalimumab (I)

- Recombinant human IgG1 monoclonal antibody specific for TNF-α
  - Created using phage display technology resulting in an antibody with human derived heavy and light chains variable regions and human IgG1:κ constant regions
  - produced by recombinant DNA technology in a mammalian cell expression

- Prescribing Information HUMIRA 2002

Immunogenicity

Patients in Studies I, II, and III were tested at multiple time points for antibodies to adalimumab during the 6 to 12 month period. Approximately 5% (58 of 1,062) of adult rheumatoid arthritis patients receiving HUMIRA developed low-titer antibodies to adalimumab at least once during treatment, which were neutralizing \textit{in vitro}. Patients treated with concomitant MTX had a lower rate of antibody development than patients on HUMIRA monotherapy (1% versus 12%). No apparent correlation of antibody development to adverse events was observed. With monotherapy, patients receiving
Example: Adalimumab (II)

Clinical Reports 2007-11

- Bartelds et al.,
  Ann Rheum Dis 2007, 66, 921-6
  - Active RA patients (n=121)
  - Either HUMIRA monotherapy or with DMARD (including MT)
  - ADA incidence week 28:
    - Humira: 17%

- Bartelds et al.,
  JAMA 2011, 305, 1460-8
  - Active RA patients (n=272)
  - Either HUMIRA monotherapy or with DMARD (including MT)
  - ADA incidence week 28:
    - Humira: 19%
  - ADA incidence week 156:
    - Humira: 28%
Example: Adalimumab (III)

Current Prescribing Information

- **RA**
  - Approximately 5% (58 of 1062) of adult RA patients receiving HUMIRA developed low-titer antibodies to adalimumab at least once during treatment, which were neutralizing in vitro.
  - Patients treated with concomitant methotrexate (MTX) had a lower rate of antibody development than patients on HUMIRA monotherapy (1% versus 12%).

- **JIA**
  - In patients with polyarticular JIA who were 4 to 17 years of age, adalimumab antibodies were identified in 16% of HUMIRA-treated patients.
  - In patients receiving concomitant MTX, the incidence was 6% compared to 26% with HUMIRA monotherapy.

- **AS**
  - In patients with AS, the rate of development of antibodies to adalimumab in HUMIRA-treated patients was comparable to patients with RA

- **PsA**
  - In patients with PsA, the rate of antibody development in patients receiving HUMIRA monotherapy was comparable to patients with RA; however, in patients receiving concomitant MTX the rate was 7% compared to 1% in RA

- **CD**
  - In adult patients with CD, the rate of antibody development was 3%
Example: Adalimumab (IV)

Adalimumab Biosimilars vs. Humira

- **BI695501**
  - Moderate-to-severe RA patients (n=593): BI695501 vs. Humira (US)
  - Stable MTX background therapy: 15-25 mg/week
  - ADA incidence week 24:
    - BI695501: 47.5% (~50% neutralizing)
    - Humira: 53.0% (~50% neutralizing)
  - ADA incidence week 24: ~50%

- **SB5**
  - Moderate-to-severe RA patients (n=508): SB5 vs. Humira
  - Stable MTX background therapy: 10-25 mg/week
  - ADA incidence week 24:
    - SB5: 32.4% (~50% neutralizing)
    - Humira: 31.4% (~50% neutralizing)
  - ADA incidence week 24: ~30%

- **ABP501**
  - Moderate-to-severe RA patients (n=494): ABP501 vs. Humira
  - Stable MTX background therapy: average 16.6-16.9 mg/week
  - ADA incidence week 26:
    - ABP501: 38.3% (~24% neutralizing)
    - Humira: 38.2% (~29% neutralizing)
  - ADA incidence week 26: ~40%
Circulating immune complexes trigger regular endogenous elimination processes

Uptake and lysosomal degradation by reticulo-endothelial system (phagocytic cells [monocytes and macrophages])
- Primarily in liver and spleen
- Mediated via Fc$_γ$ receptors, primarily Fc$_γ$RIIb2
  (in rat liver sinusoidal endothelial cells)
- Human platelets contribute to the clearance of IgG-containing complexes
  - Express Fc$_γ$RIIA
  - Bind IgG complexes and are internalized by circulating phagocytes

Huang et al., Mol Immunol 2011, 48, 691-6
Anti-Drug Antibodies

With No Effect on Disposition

Panitumumab exposure in patients with and without ADA (Median; lower and upper quartiles; 95% confidence intervals; Box widths \( \sqrt{n} \))

Ma et al., J Clin Pharmacol 2009, 49, 1142-56
Proteolysis
Renal metabolism

Proteolysis in tissue

Drug-target complex degradation

ADA immune complex formation & disposition

ADA turnover

kformation

kcat

Kd

ADA

CL4

ADA-drug complex degradation

ADA-PT

Presystemic Degradation

kdeg

D

Q

CL2

CL1

FcRn recycling

Proteolysis Renal metabolism

Target-mediated drug disposition

Receptor turnover

koff

kon

kdeg

kdeg

PT-R

CHirmule, Jawa & Meibohm. AAPS J 2012, 14: 296-302

kformation

kcat

Kd

ADA-PT

CL4

ADA-drug complex degradation

Chirmule, Jawa & Meibohm. AAPS J 2012, 14: 296-302
Challenges in ADA Assessment

- ADA assays are qualitative or semi-quantitative assessments
- Lack of defined, standardized positive controls, the polyclonal nature and between-patient variability of immune response make comparisons between different drugs and different assay platforms impossible
- Drug and target tolerance pose limitations on ADA assays
- Due to heterogeneity in ADA response in different patients semi-quantitative measurements (titer) may not be related to clinical effects
- Incidence and magnitude of ADA response as assessed by ADA assays always needs to be considered in context with its PK (clearing/sustaining) and PD (neutralizing) effects for meaningful clinical interpretation

Take-home Message for Clinical Pharmacologists
9th Introductory Pharmacometric Training Course

Pharmacokinetics & Pharmacodynamics of Protein Therapeutics
- Concepts and Hands-On Modeling and Simulation -

Course Directors:
Bernd Meibohm, University of Tennessee
Johan Gabrielsson, Swedish University of Agricultural Sciences

The 5-day course will introduce participants to basic principles in the pharmacokinetic and pharmacodynamic evaluation of novel protein therapeutics and provide opportunities for hands-on PK and PK/PD modeling and simulation examples relevant for protein drugs. Topics include target-mediated drug disposition, tissue and tumor penetration, interspecies scaling, first-in human dose selection, immunogenicity, model-based drug development, disease progression modeling, and drug-drug interactions. Hands-on data analysis will be performed individually and in small groups using several software packages.

Location: University of Tennessee College of Pharmacy, Memphis, TN, USA

Time: April 1-5, 2019

Last updated: May 30, 2012

Click here First Announcement Flyer

Participants of the 2nd 'PKPD of Protein Therapeutics' pharmacometric training course, April 2012
Memphis, Tennessee