Anti-Drug Antibody Testing Strategies for Nonclinical Toxicity Studies and Results Interpretation

Part 2: Toxicity Associated with ADA: Identification and Interpretation

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Outline

• Mechanisms of anti-drug antibody induced toxicity

• Case studies – assessing ADA-related toxicity in the nonclinical setting

• Clinical implications of nonclinical ADA toxicity
Impact of Anti-Drug Antibodies in Toxicology Studies

These effects may happen in animals or humans:

- Minimal to no effect
- Impact on toxicokinetics – increase or decrease in drug clearance/exposure
- Neutralize pharmacological action of drug
- Hypersensitivity reactions (mainly Types I and III)
- Neutralize biological activity of endogenous protein
Type I Hypersensitivity Reactions

- Anaphylaxis or anaphylactoid-like reactions
  - Anaphylaxis – IgE mediated
  - Anaphylactoid – non-IgE mediated
- Reactions occur within seconds to minutes
- Antigen + antibody bind to effector cells
- Release of mediators triggered
- Skin, respiratory tract and GI tract primarily targets
- Urticaria, edema, erythema and anaphylaxis can be observed
- Can be treated with antihistamines
# Anaphylaxis vs Anaphylactoid

<table>
<thead>
<tr>
<th>True Anaphylaxis/Anaphylaxis/Anaphylactic</th>
<th>“Pseudo-anaphylaxis/Anaphylactoid”</th>
</tr>
</thead>
</table>
| Angioedema, bronchospasm, chest pain, chill, choking, conjunctivitis, coughing cyanosis, death, edema, erythema, headache, hypo-/hypertension, nausea, pruritis, rash, rhinitis, shock, tachypnea | • Non-IgE-mediated  
• Directly triggering mast cells and basophils  
• Triggering of mast cells and basophils thru complement activation |
| • IgE-mediated (Type 1 reaction) | |
| Occurs with repeated exposure (pre-sensitization needed) | Occurs with first treatment (pre-sensitization not needed) |
| Reaction stronger upon repeated exposure | Reaction is milder or absent upon repeated exposure |
| Reaction does not cease without treatment | Reaction can resolve spontaneously |
| Reaction rate is low (< 2%) | Reaction rate higher (up to 45%) |

Type III Hypersensitivity Reactions

Immune complex induced tissue injury

- Tissues are injured by local or systemic inflammation
  - Arthus reaction (localized reaction) and serum sickness (systemic reaction)
  - Lesions in vascular walls, kidneys (glomeruli), lungs, joints, heart (arteries and endocardium), and joints

- Tissue injury is determined by the ratio of antigen (drug) and antibody (ADA)

- Reaction is mediated mainly by complement

- Neutrophils are responsible for much of the tissue damage

- The antigen (drug) may be completely innocuous
Immune Complex Pathogenicity
Ratio of Antibody:Antigen

- Antibody excess
  - Complexes tend to be large and insoluble
  - Rapidly removed by cells of mononuclear phagocyte system
- Extreme antigen excess
  - Complexes are too small to be trapped
  - Complexes lack arrangement necessary to activate complement
- Slight antigen excess
  - Complexes are the right size to be deposited in tissues

Immune complexes and ICD may only be observed at one dose level in a toxicology study because of the needed ratio
Neutralization of Endogenous Protein

• ADA directed against drug cross-react with endogenous proteins
  – Observed with some recombinant proteins
  – Not associated with monoclonal antibodies

• Consequences can be severe
  – Pure red cell aplasia following treatment with erythropoietin
    • Observed in humans but not in nonclinical studies (Schellekens-Huub, 2006)
  – Thrombocytopenia following treatment with thrombopoietin
    • Observed in Rhesus monkeys (Koren, 2002)
Immune Complex Disease – Case Study

• Monoclonal antibody that binds to a circulating target
• 6 month monkey study conducted  
  – Doses of 10, 30 and 100 mg/kg/week
• 3 animals from 30 mg/kg dose group euthanized in moribund condition  
  – All animals had received at least 4 doses of drug
• Clinical pathology, ADA, TK and histology initially collected

Case study contributed by Bora Han, Pfizer, Inc.
Clinical Pathology Findings

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Animal #1</th>
<th>Animal #2</th>
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<tbody>
<tr>
<td>WBC</td>
<td>↑2X</td>
<td>↑4X</td>
</tr>
<tr>
<td>PLT</td>
<td>↓3X</td>
<td>↓3X</td>
</tr>
<tr>
<td>NEUA</td>
<td>↑2X</td>
<td>↑4X</td>
</tr>
<tr>
<td>LYMA</td>
<td>↑2X</td>
<td>↑2X</td>
</tr>
<tr>
<td>MONA</td>
<td>↑3X</td>
<td>↑2X</td>
</tr>
<tr>
<td>BASA</td>
<td>↑17X</td>
<td>↑52X</td>
</tr>
<tr>
<td>PT</td>
<td>ALQ</td>
<td>ALQ</td>
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<td>ALQ</td>
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<tr>
<td>FIB</td>
<td>BLQ</td>
<td>BLQ</td>
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<tr>
<td>ALB</td>
<td>↓2X</td>
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<tr>
<td>AST</td>
<td>↑6X</td>
<td>↑5X</td>
</tr>
<tr>
<td>TRIG</td>
<td>↑3X</td>
<td>↑5X</td>
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</tbody>
</table>

Interpretation
- Clear evidence of systemic inflammation
- Coagulopathy suggestive of loss of glomerular antithrombin III
- Suggestive of renal disease

Total Protein (↓ 20%), BUN (↑ 30%), Creatinine (↑ 30%), Phosphorous (↑ 3X)
Other Preliminary Findings

Histopathology
• Kidneys: Mild increase in mesangial matrix of glomeruli

Toxicokinetics
• None to very low drug exposure in moribund animals
• Expected exposure in non-impacted animals

Anti-Drug Antibodies
• High levels in moribund animals
• None to low levels in non-impacted animals

Next Steps:
• Assess lesions by IHC (monkey IgG, IgM, and complement and human IgG (drug)
• Assess serum complement activation
• Electron microscopy of lesions
Complement C3 Staining in Monkey Kidney

Results conclusive
- Clear increased staining with monkey IgG, IgM, C3, SC5b-9, and test article
- Observed in kidney glomeruli
# Serum Complement Activation Product Analysis

<table>
<thead>
<tr>
<th>Animal</th>
<th>Collection Conditions</th>
<th>C3a (ng/mL)</th>
<th>C4d (ug/mL)</th>
<th>sC5b-9 (ug/mL)</th>
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</thead>
<tbody>
<tr>
<td>Controls (range, n=2)</td>
<td>Room temp.</td>
<td>548-862</td>
<td>BLQ</td>
<td>137-183</td>
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<td>Controls (range, n=6)</td>
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<td>181-481</td>
<td>BLQ</td>
<td>BLQ-143</td>
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<tr>
<td>#1</td>
<td>Banked serum</td>
<td>1018</td>
<td>10</td>
<td>&gt;ULOQ</td>
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<tr>
<td>#2</td>
<td>Banked serum</td>
<td>952</td>
<td>4</td>
<td>&gt;ULOQ</td>
</tr>
<tr>
<td>#3</td>
<td>Cold (fresh)</td>
<td>&gt;ULOQ</td>
<td>4</td>
<td>1816</td>
</tr>
</tbody>
</table>

BLQ = below limit of quantitation; ULOQ = upper limit of quantitation
Electron Microscopy of Kidney

Subepithelial electron dense deposits in glomerulus
ICD Case Study - Conclusions

• Findings suggestive of immune complex formation were observed based on clinical pathology, TK and ADA data

• Immune complex formation was confirmed by serum complement activation, ICH and EM

• ICD was considered to be the definitive cause of the morbidity and pathology
Serum Sickness – Case Study

- Single dose with 30 day observation period
  - Administered 1, 10 and 100 mg/kg IV or SC
- Routine end-points; initially planned as non-terminal
- Toxicity initially observed in one, maybe two, high-dose males

Case study contributed by Frank Geoly, Pfizer, Inc.
Clinical Onset of Illness

- Animals received single dose on study Day 1
- Well-tolerated in all animals through Day 10
- Day 11: One high dose male developed inappetance and inactivity
- Day 12:
  - Inappetance persisted, sedentary, hunched posture
  - Truncal morbilliform rash
  - Febrile (104.3°F)
- Day 13:
  - Mild unilateral epistaxis, and small amount of blood/mucus in feces
  - Febrile (102.2°F)

Clinical diagnostic rule-outs
- Allergic drug reaction (serum sickness)
- Infectious disease?
Pharmacokinetics/ADA

Serum Concentrations (µg/mL)

Onset of signs
Animal #7

ADA + Sick

ADA + Not sick

Hours
Outcome

- Animal received supportive care from days 11-22
- Fever and rash resolved spontaneously by days 14 and 16, respectively
- Food consumption remained decreased until day 19, then gradually increased
- Animal considered normal by Day 23
- Clin path returned to baseline by Day 30
- No treatment-related findings at Day 30 necropsy
- The other high dose male also had a transient period of inappetance and low fever on Days 15-17 coincident with rapid clearance of drug
Diagnosis of Serum Sickness

Diagnosis Based On:

- Timing of onset
- Type of clinical signs - Fever, rash, hemorrhage
- Evidence of acute systemic inflammatory reaction Coincident rapid clearance of the compound and anti-drug antibody formation
- Lack of intercurrent disease at post-mortem

• Presence of a high MW impurity in the drug substance may have increased immunogenicity
• Subsequent repeat-dose GLP studies were negative
Clinical “Serum Sickness” with Rituximab

- Patient started treatment with rituximab (weekly X 4 weeks) for refractory autoimmune polyneuropathy
- ~10 days after initiating treatment, the patient was hospitalized with 3-day history of fever, malaise and arthralgias
  - Severe pain with moving shoulders, elbows, wrists, knees and ankles
- Symptoms resolved with corticosteroids
- Presence of anti-rituximab F(ab)_2 fragments detected using an immunoassay

Clinical Implications of ADA-Mediated Toxicity Observed in Animals

• In general, immunogenicity in animals does not predict immunogenicity in humans
• For drugs where ADA is not observed clinically
  – Risk is low to nonexistent even if severe ADA-related toxicity was observed in the animals
  – Sensitivity of ADA assay and impact of circulating drug on assay sensitivity
• For drugs where ADA is observed clinically
  – Risk is unknown, particularly if severe ADA-related toxicity was observed in the animals
  – Risk can be lowered in clinical trials
    • Increased monitoring
    • Clinical pathology assessments
    • Real time monitoring of ADA
      – What if clinical positives are identified?
    – Well-validated, robust ADA assay will likely be needed
Conclusions

• Both animals and humans can mount immune responses to protein therapeutics
• In both animals and humans, ADA can result in no consequences or adverse effects
• ADA and associated consequences in animals are poorly predictive of the same in humans
• In absence of predictive animal models, clinical risk can be managed thru clinical trial design, enhanced clinical monitoring and prophylactic and/or symptomatic treatment