Challenges with pre-existing anti-drug antibodies

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Current analysis of immunogenicity – Best Practices and Regulatory Hurdles
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Agenda

1. Introduction to pre-existing anti-drug antibodies (pre-ADA)
2. Challenges with pre-ADA
3. Examples for assay cut point evaluation / assay strategy in case of the presence of pre-ADA
4. Summary/Considerations
1. Introduction

*Pre-existing anti-drug antibodies are:*

- Present before a subject is treated with a biotherapeutic
- Specific (e.g. anti-PEG-ABs, ABs to antibody fragments) or cross-reactive (e.g. Rheumatoid factors, heterophilic ABs)
- Either part of the natural antibody population or antibodies of an adaptive immune response to similar biotherapeutics or environmental antigens
- Potentially affecting PK, efficacy, safety, occasionally without clinical impact

Gorovits *et al*, *AAPS J*, 18(2), 2016

Found for biotherapeutics which are potentially foreign to a patient e.g. new constructs
1. Introduction

Examples for new constructs:

Humanized AB-umab

Spiess et al. / Molecular Immunology

→ growing evidence, also in literature, about existence of pre-ADA
1. Introduction

- Literature example: TAS266 - agonistic tetravalent Nanobody® targeting the DR5 receptor

1. Introduction

- Literature example: **GSK1995057** - fully human, single heavy chain variable domain (V\textsubscript{H}) antibody directed against the TNFR1 receptor

1. Introduction

- “The sponsor should identify those samples with pre-existing antibodies, ... and remove them from the cut point analysis.”
- “If the presence of pre-existing antibodies is a confounding factor, it may be necessary to assign positive responses or a cut point based on the difference between individual patient results before and after exposure.“
- “An alternative to the qualitative screening assay approach may be needed to assess the quantity and quality of ADA when pre-existing antibodies are present. For example, testing samples for an increase in ADA”
1. Introduction

- EMA Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins; Sept 2015
  - “Pre-existing antibodies against a variety of protein therapeutics are frequently encountered.”
  - “While the impact of pre-existing antibodies on safety and/or efficacy of biologics is poorly understood, consequences could be severe. Therefore, potential cross-reactivity with pre-existing antibodies should be considered.”
  - “Some individual’s/patient’s samples may contain pre-existing antibodies ... for this is necessary to ensure that post-treatment data can be interpreted correctly in terms of treatment emergent antibodies.”
  - “Evaluate impact of pre-existing Abs on pharmacokinetics, safety and efficacy”
2. Challenge

Setting up an assay strategy which:

• Distinguishes pre-ADA from false-positive results due to other interfering factors, e.g. multimeric drug target
• Avoids generation of an inappropriately high cut point
• Reduces risk of false-negative results
• Measures treatment-boosted ADA

No standard procedure exist to determine an immunogenicity assay cut point when a high prevalence of pre-ADA is observed
3. Examples for assay cut point evaluation / assay strategy in case of presence of pre-ADA

- Example 1: Usage of Immunoglobulin (Ig) depleted naive human sera for assay cut point evaluation (cell-based therapy)
- Example 2: Measuring samples without assay cut point (gene therapy)
- Example 3: Usage of drug inhibited naive human sera for assay cut point evaluation (nanobody therapy)
Example 1: Usage of Ig depleted naive human sera for assay cut point evaluation

**Challenge:**
Pre-ADA against drug were detected in majority of evaluated normal human sera.

**Possible solution:**
IgG/IgM depleted sera were used to establish the cut point factor (CPF)

<table>
<thead>
<tr>
<th>Using Ig-depleted individual sera</th>
<th>Using naive individual sera</th>
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<tbody>
<tr>
<td>Low CP: majority of individual samples which showed high signal were reported as IG positive – safe approach</td>
<td>High CP: &gt;50% of the samples which showed high signal would have been reported as IG negative – risky approach</td>
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Example 1: Usage of Ig depleted naive human sera for assay cut point evaluation

- **Cut point evaluation:**
  - Naive human Ig-depleted sera (62) were 3x analyzed
  - CPF was defined with a non-parametric approach as the 99.9th percentile

- **Result evaluation:**
  1) *Screening assay:*
     - 1\textsuperscript{st}: all samples were evaluated with the assay specific cut point
     - 2\textsuperscript{nd}: pre-dose sample was used to calculate a subject-specific cut point
  2) *Titration assay:*
     - For ADA+ subjects the pre-dose and a post-dose sample with the highest signal was titrated
  3) *Conclusion:*
     - Evaluation of potentially boosted ADA response, Evaluation for sustained or transient ADA, Conclusion on clinical significance
Example 2: Measuring samples without assay cut point

• **Challenge:**
  Literature reports pre-ADA in up to 100% of monkeys

• **Possible solution:**

  **Strategy:**
  1. No screening assay cut point factor determination
  2. ADA assessment directly in confirmatory assay
  3. Confirmed positive sample titration

Source: Lydia Michaut/ Kerstin Kentsch (Novartis) - Based on Nov-2015 presentation at EBF meeting
Example 2: Measuring samples without assay cut point

- **Cut point evaluation:**
  - No CPF was evaluated
  - Negative control pool based on sera with very low signal un-specific for the drug
  - Global confirmatory assay precision was determined (n=108): 24%

- **Result evaluation:**
  1) **Confirmatory assay:**
     - Samples were analyzed: drug-spiked and naive
     - Drug-inhibited samples compared to the same naive sample ≥ 24% -> ADA+
  2) **Titration assay:**
     - ADA+ samples titrated in 4 dilution steps
     - Titer Cut Point = 2 x plate mean negative control
  3) **Result:**
     - 89% of the samples were and analyzed in the titer assay

Source: Lydia Michaut/Kerstin Kentsch (Novartis) - Based on Nov-2015 presentation at EBF meeting
Example 3: Usage of drug inhibited naive human sera for assay cut point evaluation

• **Challenge:**
  Naive samples contained both ADA+ and ADA-

• **Possible solution:**

  **Strategy:**
  1. Selecting ADA- sera assuming a mixed distribution model for cut point determination
     Examples published
  2. ADA assessment directly in confirmatory assay based on a subject specific cut point
  3. Confirmed positive sample quasi-quantified

Source: Kerstin Kentsch, Matthias Hofmann (Novartis)
Example 3: Usage of drug inhibited naive human sera for assay cut point evaluation

• Cut point evaluation:
  – Sera were classified into ADA+ and ADA-
    Example: F. Schaarschmidt et. al.
  – Sera with the lowest signal were used for determination of CPF and confirmatory CP

Fig. 2. Histograms of 100 observations with two subgroups. a) 100 observations drawn at random from a mixture model with 2 normally distributed subgroups, (75% truly ADA−, mean = 1, variance = 0.05; 25% truly ADA+ (mean = 3, variance = 1). Dotted and dashed lines depict the underlying normal densities of the subgroups.

• Result evaluation:
  1) Confirmatory assay:
    - Result of the drug-spiked sample was disregarded in case the signal of the drug-unspiked sample was below the assay cut point
  2) Titration assay:
    - In ADA+ the immune response was quasi-quantified to assess the intensity of the ADA response.

Source: Kerstin Kentsch, Matthias Hofmann (Novartis)
Summary/Considerations

• Different ways possible to set up an IG assay for biotherapeutics with high prevalence of pre-ADA

• Immunogenicity can be related to safety, therefore avoid false negative results -> lower cut point should be preferred

• Report prevalence of pre-ADA along with treatment-boosted and treatment-induced ADA response

• Evaluate impact of pre-ADA on Immunogenicity, e.g. higher IG in subjects with vs. without pre-ADA

• Evaluate clinical impact of pre-ADA on PK, PD, safety, efficacy

• Pre-ADA should be included in the immunogenicity risk assessment
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