Some fundamentals of immunogenicity assessment and a case study

Maria Barbosa, Ph.D.

mbarbosa@cacobio.com
www.cacobio.com

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Outline

❖ Some fundamentals of immunogenicity assessment
  ✓ Introduction – basic concepts
    ✓ Humoral
  ✓ The antibody detection pyramid
  ✓ Assay selection: “fit for purpose”
  ✓ Drug inhibition of antibody assays

❖ Case study: Addressing drug tolerance of surface plasmon resonance assays for the detection of antibodies against a bispecific therapeutic protein

❖ Conclusions
❖ Reference list
❖ Discussions
Time for acronyms

- **ADA** = anti-drug antibody
  - **drug** = biotherapeutic = therapeutic protein
  - **Immunoglobulins**
- **Ig** = immunoglobulin
  - **IgG, IgM, IgE, IgD and IgA**
- **NAb** = neutralizing antibody
- **PK** = pharmacokinetics
  - “what the body does to the drug”
- **PD** = pharmacodynamics
  - “what the drug does to the body”
- **HLA** = human leukocyte antigen
- **SPR** = surface plasmon resonance
- **Gdn** = guanidine

mbarbosa@cabobio.com
Development of small molecule drugs and biotherapeutics

Small molecule drugs and Biotherapeutics

PK & PD

Pre-clinical development

Phase 1 Clinical trials

Phase 2 Clinical trials

Phase 3 Clinical trials

Biotherapeutics

ADAs

Post marketing surveillance

mbarbosa@cacobio.com
ADA responses and assays

- Animal models or patients dosed with a biotherapeutic may develop ADAs
  - Immunoglobulins of various affinities for the drug
  - Antibody affinity is a measure of the strength of the bond between a protein epitope and the antibody binding site
- Immunoglobulins (antibodies) are bifunctional molecules
  - Basic unit of 2 light chains and 2 heavy chains
- ADA assays
  - Detection of ADAs (polyclonal antibodies) in plasma or serum samples
  - Qualitative/semi-quantitative assays
ADA responses: variable consequences

- ADAs may affect safety and/or efficacy of the drug
  (Clinical safety concerns may vary from life-threatening situations to less severe infusion reactions)
- Hypersensitivity responses
- Binding ADAs
- Neutralizing ADAs
  - May bind and neutralize the pharmacological activity of the drug and the endogenous protein counterpart

Several factors may be involved in immunogenicity of biotherapeutics

- Human genetics
- Amino acid sequence
- Protein aggregation
- Impurities
- Route, dosage and frequency of administration
- Other factors
Accurate and sensitive ADA assays are required for proper assessment of antibody responses against biotherapeutics (including biosimilars and biobetters).

The ADA testing pyramid

- ADA testing: three tier
  - False negatives during screening will **not** be detected at the top of the pyramid!

Diagram:

- **Screening**
- **Confirmation**
- **NAb assay**
Selection of ADA assay: “fit for purpose”

- Several screening ADA assay formats available
  - Radio immuno-precipitation, surface plasmon resonance (SPR), Immunoassay (electrochemiluminescence, ELISA), etc.
- Safety considerations
  - Screening assay should be able to detect all antibody isotypes, particularly IgM and the different IgG isotypes
  - IgE
- Assessment of associations between patient genetics and IgG responses
  - Post-marketing; Interferon-β assays capable of detecting IgG
Different assays may detect distinct ADAs in the same sample

**Example:** Detection of anti interferon-β (IFN- β) IgG to investigate ADA associations with patient HLA types

- IgG detection; 2 screening assays used
- Results varied with assay (direct or indirect capture of IFN- β)
- 39 samples total; only results for 32 samples agreed (they were both positive of both negative for the presence of IgG)

High drug concentrations in samples may inhibit ADA assays

Patients or animals are dosed with high concentrations of the drug

Patients or animals mount ADA responses

The serum or plasma samples will have both the ADAs and drug

Drug present in the samples may form complexes with ADAs and inhibit ADA detection
High drug concentrations in samples may inhibit ADA assays

- False negatives

- ADA assay drug tolerance needs to be consistent with drug levels in the test samples
  - Ideally assay positive controls should reflect sample ADAs

- Acid dissociation of drug-ADA complexes has been used to increase drug tolerance of ADA assays
  - Acid dissociation may denature ADAs
  - Might not dissociate all drug-ADA complexes
Sample collection should be planned to minimize drug present in ADA samples

- ADA assay drug inhibition is a particular concern for samples from patients or animals receiving high doses of biotherapeutics with long half-lives
- Sample collection prior to initiation of treatment and prior to dosing (and if appropriate after end of treatment)
- Case-by-case decisions on number and frequency of samples
Drug inhibition of ADA assays complicate evaluation of de-immunization strategies

<table>
<thead>
<tr>
<th>DRUG</th>
<th>CHARACTERISTICS</th>
<th>ADA</th>
<th>TROUGH DRUG LEVELS</th>
<th>ASSAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erbitux (cetuximab)</td>
<td>Chimeric mAb</td>
<td>49/1001</td>
<td>41-85 µg/ml</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herceptin (trastuzumab)</td>
<td>Humanized mAb</td>
<td>1/903</td>
<td>79 µg/ml</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(&lt;1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vectibix (panitumumab)</td>
<td>Fully human mAb</td>
<td>3/613</td>
<td>39 ± 14 µg/ml</td>
<td>***Acid dissociation ELISA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(&lt;1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vectibix (panitumumab)</td>
<td>Fully human mAb</td>
<td>28/613</td>
<td>39 ± 14 µg/ml</td>
<td>***BIACORE®</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4.6%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Drug trough concentrations and ADA incidence extracted from the product labels
*ADA tested with either a double antigen radiometric assay or an ELISA assay
**ADA detection method not indicated in the product label

CASE STUDY
Addressing drug tolerance of surface plasmon resonance assays for the detection of antibodies against a bispecific therapeutic protein

Surface Plasmon Resonance (SPR) ADA assays

- SPR assays detect both low and high affinity of binding ADAs

- In the presence of high drug concentrations SPR may preferentially detect ADAs with lower affinity for the drug

- Alter drug tolerance of SPR ADA assays by decreasing the affinity of ADAs for the drug
  - Temperature / chaotropic agents

ADA = Anti-drug antibodies

mbarbosa@cacobio.com
Test system: The drug, SPR assay and ADAs

**DRUG:** Bispecific tandem Adnectin (V-I-Adnectin; +/-PEG)

**SPR ASSAY:** V-I-Adnectin or PEG-V-I-Adnectin immobilized

Anti PEG-V-I-Adnectin Polyclonal antibodies

PEG-V-I-Adnectin

V-Adnectin (VEGFR2)

Anti-V monoclonal antibody (mAb-V)

PEG 40

I-Adnectin (IGF-1R)

Anti-I monoclonal antibody (mAb-I)

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Anti-PEG-V-I-Adnectin polyclonal ADAs: samples from pre-clinical study

- Cyno polyclonal anti-PEG-V-I-Adnectin ADAs (pAb-V-I)
  - Pooled plasma samples
- Monkey study plasma samples (PEG-V-I-Adnectin study)
  - H, M, L and BLQ
Binding of anti-V-I-Adnectin antibodies to immobilized PEG-V-I-Adnectin

 ADA concentration= 0.5 μg/ml

Response (RU)

Time (s)

mAb-V

mAb-I

Anti-GS

mbarbosa@cacobio.com
Testing treatments to weaken affinity of the drug-ADA interactions

**mAb-V** ; PEG-V-I-Adnectin immobilized

- Low pH (4.5, 5.0)
- Increase of temperature (30°C, 37°C)
- Guanidine hydrochloride (Gdn)
Increased drug tolerance of an ADA SPR assay with Gdn

0.5 µg/ml mAb-V; PEG-V-I-Adnectin immobilized
100 % Maximum Response = Control (pH 7.4, 25°C) without drug added

Nonspecific binding observed at pH 4.5 and 5
Binding affinity (K_D) of anti-V-I-Adnectin antibodies to PEG-V-I-Adnectin

<table>
<thead>
<tr>
<th></th>
<th>25°C</th>
<th>32°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>mAb-V</td>
<td>0.93 nM</td>
<td>1.06 nM</td>
<td>1.19 nM</td>
</tr>
</tbody>
</table>

- PEG-V-I-Adnectin immobilized
- mAb-V concentrations ranged from 0.23 to 15 µg/ml
Gdn affects binding and dissociation kinetics of mAb-V at 25°C

- PEG-V-I-Adnectin immobilized
- mAb-V concentrations ranged from 0.23 to 15 µg/ml

Buffer

\[ K_D = 0.933 \text{ nM} \]

Buffer + 1 M Gdn

\[ K_D = 348 \text{ nM} \]
Working dilution determination and background subtraction for pAb-V-I

*Cyno polyclonal anti-PEG-VI-Adnecting antibody (pAb-V-I) = pooled plasma samples; Same final plasma concentration for all dilutions*

**PEG-V-I-Adnectin binding response**

- PEG-V-I-Adnectin
  - Time (s)
  - Response (RU)
  - Sample Dilution Factor (1:X)
  - SPR Response (RU)

**V-I-Adnectin binding response**

- V-I-Adnectin
  - Time (s)
  - Response (RU)
  - Sample Dilution Factor (1:X)
  - SPR Response (RU)

Plasma background is subtracted
Drug tolerance of SPR assay with pAb-V-I

V-I-Adnectin immobilized

PEG-V-I-Adnectin immobilized

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Effect of guanidine (Gdn) on ADA-positive pre-clinical study samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>PEG-V-I-Adnectin (µg/ml)</th>
<th>Ab titer</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLQ</td>
<td>0</td>
<td>10,000-100,000</td>
<td>1:60</td>
</tr>
<tr>
<td>L: Low drug</td>
<td>11</td>
<td>10,000-100,000</td>
<td>1:60</td>
</tr>
<tr>
<td>M: Medium drug</td>
<td>60</td>
<td>10,000-100,000</td>
<td>1:60</td>
</tr>
<tr>
<td>H: High drug</td>
<td>719</td>
<td>10,000-100,000</td>
<td>1:60</td>
</tr>
</tbody>
</table>

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Pre-clinical study sample (BLQ) ADAs preferentially bind to I-Adnectin

**BLQ:** Plasma sample containing cyno polyclonal anti-PEG-V-I-Adnectin antibodies; BLQ has PEG-V-I-Adnectin concentration below limit of quantification
Concluding remarks

• A higher SPR assay signal was observed with a non-PEGylated V-I-Adnectin immobilized than with a PEG-V-I-Adnectin
  ❖ Higher V-I-Adnectin density on the chip
• Gdn increased the drug tolerance of a SPR ADA assay using an anti-V-Adnectin high affinity antibody
  ❖ Binding kinetics showed that Gdn weakened affinity of drug-antibody interaction
• The drug-antibody complexes responded differently to Gdn
  ❖ While it may be effective to weaken drug interaction with some antibodies, it may have deleterious effects on others
• In many situations a combination of approaches may be needed for accurate ADA detection
References
(Web sites last accessed on August 2013)

References (continued)


- Barbosa, M.D.F.S. 2011 Immunogenicity of biotherapeutics in the context of developing biosimilars and biobetters. Drug Discovery Today. 16: 345-353
