Immunogenicity of Biological products

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Agenda

- Introduction
- Immunogenicity monitoring
  - Guidelines, white papers…
  - Risk management plan
  - Bioanalytical tools
    - Assays platform
    - Assays development/validation
- Cases study
Different denominations

Biologics, biotechnology products, biological products, recombinant proteins, biopharmaceuticals, protein therapeutics, protein drugs, biotherapeutics…

different denominations may be encountered!
Official definitions of Biologicals

- **EMEA guidance**
  « *biological/biotechnology-derived proteins*...proteins and polypeptides, their derivatives and products of which they are components, e.g. conjugates »

- **ICH topic S6**
  « *Products derived from characterized cells* through the use of a variety of expression systems including bacteria, yeast, insect, plant and mammalian cells... proteins and peptides, their derivatives and products of which they are components; they could be derived from cell cultures or produced using recombinant DNA technology including production by transgenic plants and animals »

- **Directive 2003/63/EC**
  Substance which is produced by or extracted from a biological source and that needs for its characterization and the determination of its quality a combination of physicochemical-biological testing, together with the production process and its control.
Biologicals vs small molecules

**Small molecules**
- Organic synthesis
- Low MW (Rule of <5kDa)
- Well-defined properties
- Purity standards well established
- Optimized by medicinal chemistry

**Biologicals**
- Produced by living host cells
- Complex production process that contributes to the definition of the drug substance (DS)
- High MW (usually from 5 to 150kDa and higher)
- Complex and poorly defined properties (e.g., tertiary structures, glycosylation)
- Broad specifications that may vary during development, difficult to standardize
- Protein engineering required
### Types of biotech-products

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
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<tbody>
<tr>
<td><strong>Hormones</strong></td>
<td>Growth hormone, insulin (analogues) and erythropoietin</td>
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<tr>
<td><strong>Blood products</strong></td>
<td>Albumin, thrombolytics, fibrinolytics and clotting factors</td>
</tr>
<tr>
<td><strong>Cytokines and growth factors</strong></td>
<td>Interferons, interleukins and colony-stimulating factors</td>
</tr>
<tr>
<td><strong>Antagonists/inhibitors</strong></td>
<td>Soluble receptors</td>
</tr>
<tr>
<td><strong>Monoclonal antibodies and related products</strong></td>
<td>Mouse, chimeric or humanized Ab; whole molecule or fragment; single chain or bispecific; and naked or conjugated</td>
</tr>
<tr>
<td><strong>Modified human proteins</strong></td>
<td>Fusion (IgFc), polyethyleneglycol (PEG)ylation, liposome encapsulation and drug–toxin conjugate</td>
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<tr>
<td><strong>Vaccines</strong></td>
<td>Recombinant proteins or peptides, DNA plasmid and anti-idiotypic</td>
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<td><strong>Gene-transfer products</strong></td>
<td>Viral and non-viral vector-delivery systems and DNA–RNA chimaeras</td>
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<td><strong>Cell-based therapies</strong></td>
<td>Autologous, allogeneic and xenogeneic</td>
</tr>
<tr>
<td><strong>Tissue-engineered products</strong></td>
<td>Cells, tissues, naturally occurring/synthetic biomaterials, extracorporeal and long-term implants</td>
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It is assumed that most or all therapeutic proteins may induce an immunogenic response with production of Anti-Drug Antibodies (ADA) in patients.

Many factors contribute to immunogenicity:
- Foreign amino acid sequences
- Aggregated, oxidated, deamidated product
- Host cells proteins, manufacturing changes
- Formulation, route of administration (SC > IP > IV) and frequency of dosing
- Immune status, age, disease of patient

This immunogenicity can be in some cases associated with serious adverse effects:
- No observed effect or clinical event
- Altered PK/PD (increased or decreased exposure)
- Decreased efficacy (decrease exposure or neutralization of the product)
- **Severe hypersensitivity reactions (HSR)**
- **Cross-reactivity with endogeneous proteins, autoimmunity**
Monitoring is mandatory!

- Both biopharmaceutical industry and regulatory agencies keep on searching for more informative antibodies assays and antibody monitoring strategies.

- There is a need to assess/measure immunogenicity
  - It is a safety concern (risk-based)
  - Regulatory expectations are regularly increased
Introduction

Immunogenicity monitoring
- Guidelines, white papers…
- Risk management plan
- Bioanalytical tools
  - Assays platform
  - Assays development/validation

Cases study
### Guidelines for Biologicals

**Specific part dedicated to IMMUNOGENICITY**

<table>
<thead>
<tr>
<th></th>
<th>QUALITY</th>
<th>SAFETY</th>
<th>EFFICACY</th>
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<td>M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials (2008)</td>
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<td>Requirements For First-in-man Clinical Trials For Potential High-risk Medicinal Products (Draft 2007)</td>
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<td>Comparability of Biotechnology-Derived Medicinal Products after a change in the Manufacturing Process - Non-Clinical and Clinical Issues (2007)</td>
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AAPS Immunogenicity Sub-Committee (Biotech scientists and FDA representatives)
- review ADA testing methods across biotech industry, summarize industry experience and publish recommendations for
  - assay design/optimization
  - validation
  - testing strategies

Different « White papers » as recommendations for immunogenicity evaluation
Recommendations for ADA assays

Standardization

Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products

Anthony R. Mire-Sluis\textsuperscript{a,*}, Yu Chen Barrett\textsuperscript{b}, Viswanath Devanarayan\textsuperscript{c}, Eugen Koren\textsuperscript{d}, Hank Liu\textsuperscript{e}, Mauricio Maia\textsuperscript{f}, Thomas Parish\textsuperscript{g}, George Scott\textsuperscript{h}, Gopi Shankar\textsuperscript{i}, Elizabeth Shores\textsuperscript{j}, Steven J. Swanson\textsuperscript{d}, Gary Taniguchi\textsuperscript{k,†}, Daniel Wiera\textsuperscript{l}, Linda A. Zuckerman\textsuperscript{m}

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Received 14 March 2004; accepted 14 June 2004
Recommendation for validation of ADA assays

Recommendations For The Validation Of Immunoassays Used For Detection Of Host Antibodies Against Biotechnology Products

Gopi Shankar¹, Viswanath Devnarayan², Lakshmi Amaravadi³, Yu Chen Barrett⁴, Ronald Bowsher⁵, Deborah Finco-Kent⁶, Michele Fiscella⁷, Boris Gorovits⁸, Susan Kirschner⁹, Michael Moxness¹⁰, Thomas Parish¹¹, Valerie Quarmby¹², Holly Smith¹³, Wendell Smith¹⁴, Linda A. Zuckerman¹⁵ & Eugen Koren¹⁶.

J Pharm Biomed Anal. 2008 Dec 15
Risk Management Plan (RMP)

- Even if providing a background and data with
  - project attributes, difference between product and endogenous counterpart, Literature reference (e.g. knockout animals)
  - preclinical animal data
    - how animal modeling reflects clinical situation
    - how « good » is the assay for immune monitoring

- Both FDA and EMEA want a risk management plan for immunogenicity in submission dossier
  - RMP provides an immunogenicity risk class designation for the compound and recommends an immunogenicity testing strategy for non-clinical and clinical studies
  - RMP is a dynamic process and requires periodic evaluations with updates with relevant information
Classify the biological regarding its risk category

Risk assessment must be carried out in collaboration with toxicologists, clinicians, PK and assay experts

The greater the risk, the more extensive and more frequent Ab testing and characterization should be applied

Recommendation for routine monitoring of changes in clinical response and linking immunological findings to clinical events

Immunogenicity as part of all clinical trials

Evaluate in all patients

Analyse AE and possible link to unwanted immune response
Introduction

Immunogenicity monitoring
- Guidelines, white papers…
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Cases study
Bioanalysis support for immunogenicity monitoring

- Development and validation of different assays for
  - Binding ADA (anti-Drug Antibody) evaluation in preclinical and clinical studies
  - ADA Characterization
  - + PK assay (complementary assay to ADA assay)

- Using different technologies
  - Select the more appropriate assay (regarding specificity, sensitivity, high throughput method...)

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3 assays are expected for immunogenicity evaluation

Minimum Requirements

1. Screening with cut-off approach
2. Confirmatory
3. Characterization
   【(Titration, Neutralization, Isotyping)

Screening assays are the first pass at detecting anti-drug antibodies.

Since it is expected that 5% false positives will be detected, a confirmatory assay is used to discount the false positives.

All confirmed positive samples must be titrated and assessed for their neutralizing activity (isotyping may be required in some cases).
Process for immunogenicity monitoring

- **Screening assay (1)** (cut-off approach)
  - Positive
    - **Confirmatory assay (2)**
      - competition test with drug in screening assay (common approach)
        - Positive
          - Neutralizing assay (3)
            - bioassay based on MOA
              - Titre or concentration
                - Isotype determination
          - Correlation? with clinical observations
        - Negative
      - Negative
  - Negative

**FINAL**
Antibody binding to the biological (ADA) can be monitored by:

- Radio-Immunoprecipitation (RIP)
- Direct / indirect ELISA
- Bridging ELISA
- Electrochemiluminescence (ECL)
- Surface plasmon resonance (Biacore)
- Magnetic bead LC/MS

Bioassays investigating neutralizing effects of the antibodies
Direct ELISA

**Assay principle**

**Pros:**
- Sensitivity
- Commercially available secondary antibodies
- High throughput

**Cons:**
- Source of the positive control has to be the same as that of the anti-drug antibodies
- Specificity (unspecific binding to matrix components)
- Can miss low-affinity antibodies due to the high number of washing steps
Bridging ELISA

**Format-1**

1. Streptavidin is coated on the plate
2. Coated plate is blocked
3. Biotin-conjugated drug binds to streptavidin
4. ADA in test sample binds to biotin-conjugated drug
5. Enzyme-conjugated drug binds to ADA and produces signal after substrate is added.

**Legend**

- Drug molecule
- Biotin-conjugated drug molecule
- Anti-drug antibody (ADA) molecule
- Streptavidin molecule
- Enzyme-conjugated streptavidin molecule
- Enzyme-conjugated drug molecule

**Format-2**

1. Drug is coated on the plate
2. Coated plate is blocked
3. ADA in test sample binds to drug
4. Biotin-conjugated drug binds to ADA
5. Enzyme-conjugated streptavidin binds to biotin-conjugated drug and produces signal after substrate is added.

*Dong Geng* 2004 *J. Pharm. and biomedical analysis*
Bridging ELISA

Pros:
- High throughput
- Specificity (two-fold binding of drug required for signal)
- Possibility to use any positive control from different origin since format is species independent
- Same format can be used for both pre-clinical and clinical!

Cons:
- Sensitivity (special orientation of coated drug required) may be limitant
- Detection of low-affinity antibodies may be restricted
- Biotinylation might mask/denature epitopes recognized by anti-drug antibodies
Drug coated to sensorchip
Injection of plasma / serum containing anti-drug antibodies
Enhance the signal with anti-species Ab
**Pros:**
- Large dynamic range
- Secondary reagents not mandatory/ not species dependent
- Detection of low affinity antibodies
- Sensorgrams include information about affinity of anti-drug antibodies
- Easy procedure for isotyping (IgG, IgA, IgM, IgE)

**Cons:**
- Masking of binding epitopes by chemical coupling
- Less sensitive than ELISA (May be superior to ELISA in detection of low affinity ADAs in certain circumstances)
- Time consuming, usually not adapted for high throughput screening
- Costs (specific equipment)
Bioassay for Neutralizing Ab detection

Ideally, should be based on MoA of the drug!

Human Receptor expressing cell
Intracellular signal (cAMP or other)
induced by Ligand and
Inhibited by Drug

Neutralizing antibodies evaluation

No NAb

Presence of NAb
Introduction

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Cases study
**Cut-off definition**

**Screening assay**

- Defined as the level of response of the ADA assay at and above which a sample is defined to be « reactive » (« potential positive ») for the presence of ADA and below which it is probably negative.

- One of the main validation item for ADA assay.

- Is established by a statistical evaluation of responses for a set of samples (~50-100) representative of naïve animals / subjects (negative for ADA).
Determination of the cut-off

- Qualitative assay with cut-off approach statistically determined providing 5% false positive rate (mean + 1.645 SD for normal distribution or 95th percentile)

- Normalisation factor (NF)
  \[ \text{NF} = \frac{\text{relative response Cut-Off}}{\text{relative response of negative control pool}} \]

- For each plate calculation of Normalised Cut-off
  \[ \text{Normalised Cut-off} = \text{NF} \times \text{relative response of negative control} \]
5% false positive rate is recommended

It is more appropriate to have false positive than false negative (when using a risk based approach)

- White Paper recommends Mean ± 1.645 SD rule
- ~5% of neg population should test positive
- Hopefully - no false negatives
Some other recommendations for cut-off...

- Use samples from an appropriate population for the cut-off determination

- Start with healthy subject plasmas then re-define cut-off with individual patient plasmas as soon as available (clinical program progresses beyond Phase I or target disease population is available)

- It is recommended to use at least 50 (15-20 for animal) different naives human samples for cut-off determination

- It is established on 3 independent runs
ADA assay sensitivity determination

Sensitivity

- Defined by the lowest concentration at which a positive control antibody preparation provides a positive signal
  - Providing sensitivity of the assay
- Sensitivity is highly dependant of the positive control (affinity, avidity, etc)
- Sensitivity of the assay must be expressed in concentration limits (mass of ADA / volume unit)

**Target Sensitivity:**
- < 250ng/mL for human
- < 500-1000ng/mL for animal

### Diagram

- **Serial dilution of positive control**
- **Cut off**
- **ADA concentration**
- **Signal**

**i.e.** 10 µg/mL

**i.e.** Sensitivity=250 ng/mL
Establish a suitable system of control to ensure the validity of results
- A QC set must be defined and a recommended one would include: Negative QC / QC near the cut point / LOW QC / HIGH QC
- Define acceptance criteria for QCs
- QC set will be used to validate each run
ADA assay validation

- Cut-off determination
- Sensitivity and Specificity
- Free Drug interference
- Matrix effect
- Stability of positive control and incurred samples (when available)
  - 24h @37°C, -20°C (-80°C) for months
  - Freeze/thaw cycles
- Dilution and parallelism
- Co-medications…
Main challenges of immunogenicity monitoring

- Matrix effect, impact on sensitivity

- Interference of residual drug in samples
  - Proteins, particularly Mab, have long $\frac{1}{2}$ life (2-3 weeks)
  - May lead to underestimation of ADA
  - Need optimization of assay to reduce interference
  - Design studies so that late time-points are collected for monitoring ADA

- ADA follow-up
  - from preclinical to late clinical phase III and then post registration to ensure long term safety and efficacy
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Cases study
Case study 1 – Therapeutic Protein X

- Drug = Tetramer protein (monomer of 34 kDa) produced in yeast
- One cycle treatment: 5 i.v. administrations over a week
- Immunogenicity evaluation:
  - A first qualitative assay was validated for ADA monitoring
    - Direct ELISA: Protein X coated plate / plasma incubation / anti-hIg -HRP
    - Cut-off determination for each assay
    - Assay for IgA, IgM, IgG detection

- FDA required two distinct and quantitative assays for IgG and IgE anti-protein X detection
  - Mab anti-protein X was chimerized into human IgG and human IgE
  - Development and validation of 2 « semi-quantitative » assays
Case study 1 - IgG assay

- IgG assay format: Direct ELISA
  - Protein X coated plate / unknown plasma or chimeric Ab spiked plasma (Std) / anti-hIgG –HRP
- N cut-off determination on 100 healthy subject plasmas
- N cut-off confirmation on 50 patient plasmas
  - N cut-off = 1.95
- Clinical samples analysis
  - Qualitative approach with cut-off determination for each plate
  - + Semi quantitative approach with validated calibration range: 0 to 10 µg/mL (with QCs at 0.6 / 1.8 / 5 and 10µg/mL)

![Cut-off + calibration curve](image)

Cut-off = 1.95 X response of pool negative
Case study 1 – IgE assay

- IgE assay format: EIA
  - anti-hIgE coated plate / unknown plasma or chimeric IgE Ab spiked plasma (Std) / Drug X conjugated to biotin / streptavidin-HRP
- N cut-off determination on 100 healthy subjects plasmas
- N cut-off confirmation on 50 patient plasmas
  - N Cut-off = 1.46

Clinical samples analysis
  - qualitative with cut-off determination for each plate
  - + semi quantitative with calibration range 0-20ng/mL with LLOQ of 2ng/mL
SSR29261: extractive avidin purified from hen egg proteins

This protein may be used as neutralizing agent for biotinylated anticoagulant molecule

Thanks to the very high affinity between Biotin and Avidin: $K_d = 10^{-15}$
## Case study 2 - Immunogenicity risk evaluation

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Risk category</th>
</tr>
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<tbody>
<tr>
<td><strong>Ig anti-avidin antibodies</strong></td>
<td>High</td>
</tr>
<tr>
<td>High MW (~64kDa) exogeneous (extracted from hen egg) protein</td>
<td></td>
</tr>
<tr>
<td>I.V. administration</td>
<td>Low-medium</td>
</tr>
<tr>
<td>Low frequency of dosing (in routine)</td>
<td>Low</td>
</tr>
<tr>
<td><em>Use only for neutralization of biotin-anticoagulant in case of over-bleeding or to stop anticoagulant for surgery (= one or two occasions)</em></td>
<td></td>
</tr>
<tr>
<td><strong>Allergic reaction and IgE anti-avidin antibodies</strong></td>
<td>Medium</td>
</tr>
<tr>
<td>Presence of natural anti-avidin antibodies in human, risk of hypersensitivity reaction?</td>
<td></td>
</tr>
<tr>
<td><strong>Neutralizing anti-avidin antibodies</strong></td>
<td>Very low</td>
</tr>
<tr>
<td>High affinity of avidin for biotin (Kd :10^{-15}) compared to Ag-Ab (mean Kd :10^{-9})</td>
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</tbody>
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Conclusions

- Immunogenicity monitoring is mandatory for all biological products (peptides, proteins, Abs, conjugates..)

- Process strategy (screening + confirmatory + characterisation) is well defined

- But each biological is unique and requires specific assays for ADA and NAb detection

- Validation of ADA assay is challenging and is not standardized

- Immunogenicity = laboratory observations + clinical observations + PK + safety + efficacy..
ACKNOWLEDGEMENTS

- Bioanalysis group of Montpellier
- SpiBio team
- I. Paty and F. Berard
- AAPS sub-comitee