Neutralising Assay Methodologies

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EMA workshop on immunogenicity assessment of biotechnology derived therapeutic proteins

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## Key Points About Neutralising Antibodies

<table>
<thead>
<tr>
<th>1. Definition</th>
<th>• These types of anti-drug antibodies (ADAs) interfere with the <strong>biological activity</strong> of the drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Impact of NAbs to patient</td>
<td>• Could range from none to serious impact depending upon the magnitude of NAbs produced resulting in reduced exposure and/or efficacy</td>
</tr>
</tbody>
</table>
| 3. NAb testing strategy | • 2\textsuperscript{nd} tier of immunogenicity assays  
• NAb test is usually preceded by a binding ADA test |
| 4. NAb methodologies | • Functional *in vitro* serum-based assays capable of assessing change in the biological activity of the drug  
• Cell-based  
• Non cell-based (Ligand binding)  
• Qualitative determination |
| 5. Characterization of NAbs | • Titer (serial dilution of sample/fixed drug conc)  
• Quasi-quantitative determination |
NAb Assay Methodologies

**Goal : To detect clinically relevant NAb**

<table>
<thead>
<tr>
<th></th>
<th>Cell based</th>
<th>Non cell based</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pros</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physiological Response of drug</td>
<td>Complex</td>
<td>Simple and binding ability of drug is measured</td>
</tr>
<tr>
<td>Is measured</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cons</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complex</td>
<td>Simple</td>
<td></td>
</tr>
</tbody>
</table>

**Assay characteristics : Sensitivity, specificity, drug product tolerance, reproducibility**

The decision to use a cell-based or non cell based NAb assay is inconsistent across industry.
Direct Cell-Based Assay Measuring Proliferation

- Cells only
- Cells + Drug
- Cells + Drug + Anti-Drug Ab

Assay Response
- Baseline
- Inhibition of Proliferation
Direct Competitive Ligand Binding Assay

Instead of cells, the assay uses purified preparation of the target protein that the drug binds to
<table>
<thead>
<tr>
<th>NAb Assay Format</th>
<th>Matrix interference Assay Format</th>
<th>MIA Type</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Direct Cell Based</strong></td>
<td>Effect of sample on cell response</td>
<td>ALTERNATIVE STIMULUS</td>
<td>Cytokines, Growth Factors, agonistic MAbs</td>
</tr>
<tr>
<td><em>(uses a factor-dependent cell line)</em></td>
<td>by another stimulus</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Indirect Cell Based</strong></td>
<td>Effect of sample on cell response</td>
<td>SAMPLE INDUCED RESPONSE</td>
<td>MAbs, soluble receptors</td>
</tr>
<tr>
<td></td>
<td>in absence of added drug and/or ligand</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Direct or Indirect Cell based or Non cell based</strong></td>
<td>Sample pretreatment with protein G/L followed by test in NAb assay</td>
<td>IMMUNO DEPLETION</td>
<td>All types of biological therapeutics</td>
</tr>
</tbody>
</table>
## Need for a consistent NAb assay selection strategy across Industry

<table>
<thead>
<tr>
<th>Regulatory Agency</th>
<th>Year</th>
<th>Type of Biological Therapeutic/Area</th>
<th>Guidance for NAb assay Format</th>
<th>Additional text</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMA</td>
<td>2007</td>
<td>Biotechnology-derived therapeutic proteins</td>
<td>Functional bioassay</td>
<td>Adaptation of potency assay</td>
</tr>
<tr>
<td>EMA</td>
<td>2012</td>
<td>Monoclonal Antibodies</td>
<td>Competitive ligand binding assays</td>
<td>Cell based assays may offer an advantage</td>
</tr>
<tr>
<td>FDA (draft)</td>
<td>2009</td>
<td>Therapeutic proteins (Assay Development)</td>
<td>Cell based bioassay</td>
<td>Should reflect the product’s mechanism of action</td>
</tr>
<tr>
<td>FDA</td>
<td>2014</td>
<td>Therapeutic proteins (Immunogenicity assessment)</td>
<td>Appropriate immunogenicity assays should be implemented</td>
<td>Impact of NAbs on life saving drugs</td>
</tr>
</tbody>
</table>
# AAPS LBABFG/Immunogenicity Assay Working Group Committee

<table>
<thead>
<tr>
<th>Name</th>
<th>Company</th>
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</thead>
<tbody>
<tr>
<td>Shalini Gupta, Chair</td>
<td>Amgen</td>
</tr>
<tr>
<td>Renuka Pillutla</td>
<td>Bristol Myers Squibb</td>
</tr>
<tr>
<td>Yuanxin Xu</td>
<td>Genzyme, Sanofi</td>
</tr>
<tr>
<td>Xu-Rong Jiang</td>
<td>MedImmune, Astra Zeneca</td>
</tr>
<tr>
<td>Shan Chung</td>
<td>Genentech</td>
</tr>
<tr>
<td>Manoj Rajadhyaksha</td>
<td>Regeneron</td>
</tr>
<tr>
<td>Joleen White</td>
<td>EMD Serono</td>
</tr>
<tr>
<td>Jim McNally</td>
<td>EMD Serono</td>
</tr>
<tr>
<td>Bonnie Wu (Lead Author)</td>
<td>Janssen, J&amp;J</td>
</tr>
<tr>
<td>Joao Pedras-Vasconcelos</td>
<td>FDA</td>
</tr>
</tbody>
</table>
White Paper (in draft)

• Title
  – Strategies to Determine Assay Format for the Assessment of Neutralizing Antibody Responses to Biotherapeutics

• In-Scope
  – Provide guiding principles for decision making strategy to use a cell-based or non cell-based NAb assay

• Out of Scope
  – Recommendation to include/exclude NAb testing in immunogenicity strategy

• Timing
  – Paper submission to Journal targeted by Q1 2016
Considerations for selection of NAb assay format

Therapeutic MoA
Examples:
• Agonists
• Antagonists
• Multiple domain biotherapeutics
  - Multi-specific biotherapeutics
  - ADCs
  - Effector function mAbs
• Enzyme biotherapeutics
• Etc.

Primary Determinant
(Cell-based vs Non Cell-based Assay?)

Assay Performance Characteristics
• Sensitivity
• Specificity
• Selectivity
  - drug tolerance
  - target tolerance
• Precision
• Robustness
• Etc.

Risk Assessment
• High risk biotherapeutics
  - high risk to patient mediated by NAbs
• Low to medium risk biotherapeutics
  - Moderate and manageable risk

Indicators of Assay Reliability

For Shaping the Assay Expectations

A reliable NAb assay should be able to detect clinical relevant NAbs.
Biologic Therapeutics Categorized by MoA

Agonist
- Antibody Drug Conjugate (ADC)
  - Cell-based assay
    - ADC delivers drug into cells
- Cell-based functional assay

Antagonist
- Therapeutic Antibody with Effector Function
  - Recommended
  - Cell-based effector assay
  - Other acceptable formats
    - Cell-based binding assay and/or non-cell based CLBA
- Enzyme Therapeutic
  - Enzyme functions in circulation
  - Non-cell based assay
    - Cell-based assay or non-cell based assay
  - Other acceptable formats
    - Cell-based assay or non-cell based assay

Antibody Drug Conjugate (ADC)
- Soluble receptor
  - Therapeutic competitively blocks receptor
  - Cell-based assay or non-cell based CLBA

Therapeutic Antibody with Effector Function
- Thrombin receptor
  - Therapeutic competitively blocks receptor
  - Cell-based assay preferred; CLBA acceptable

Bispecific drug will follow the decision tree for each targeting entity

USP Chapter 1106
Bioanalytical Perspective
On Trigger-based NAb testing

- Regulatory input is important for this approach
- The “trigger” needs to be well-defined
- Bioanalytical efforts are still needed to prepare if a clinical “trigger” is observed
  - NAb assay format selection
  - Availability of a NAb positive control
  - Level of assay development, qualification and/or validation needs thought
- NAb+/- results originating from a NAb test enable easier study interpretation
Conclusions

• NAb assays are qualitative assays

• A well-designed NAb assay should be able to detect clinically relevant NAbs

• Efforts are ongoing in industry to compile recommendations for consistent use of cell-based and non cell-based NAb assays

• Bioanalytical approaches for “Trigger”-based NAb assay availability need discussion