Antibody Arrays for Biosimilar and Novel mAb Higher Order Structure Comparability Analysis

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Topics Covered Today

1. Why a New Technology for Protein Conformational Analysis
2. Technology Development
3. Applications in Novel and Biosimilar mAb Conformational Analysis
4. Conclusions
1. Why a New Technology for Protein Conformational Analysis
Studies Demonstrating the Importance of 3-D Structure and Its Stability for Immunogenicity

• Laat, B. et al. 2011. Immune responses against domain I of β2-glycoprotein I are driven by conformational changes. Arthritis & Rheumatism. 63(12)3960-3968.
Studies and Review Papers on Therapeutic Protein Immunogenicity

The FDA guidance further stated that “The three dimensional conformation of a protein is an important factor in its biological function. Protein generally exhibit complex three-dimensional conformations (tertiary structure and, in some cases, quaternary structure) due to their large size and the rotational characteristics of protein alpha carbons. The resulting flexibility enables dynamic, but subtle, changes in protein conformation over time, some of which may be absolutely required for functional activity.” “...... at the same time, a protein’s three-dimensional conformation can often be difficult to define precisely using current physiochemical analytical technology.”
Current Technologies for Conformational Analysis

• Near UV CD Spectrum
• Size Exclusion Chromatography (SEC)
• Analytical Ultracentrifugation (AUC)
• Non-denaturing Electrophoresis
• Bioassays
• NMR
• Hydrogen/deuterium exchange (HDX)

A more sensitive and high throughput technology is desirable to investigate protein conformational changes, especially in monoclonal antibodies.
2. Technology Development
Protein Promiscuity: the short-chain dehydrogenase/reductase proteins. Superposition of 15 short-chain dehydrogenase/reductase protein structures based on their Cα atoms; two different views.
Technology Development

Individual peptides to raise Polyclonal antibodies.

Antiody amino acid sequence is used to design the antibody array with overlapping regions to cover the whole mAb molecule.
This antibody array technology measures the mAb surface **Linear Epitope** Exposure and Some **Secondary Structure-derived Epitope** Exposure, providing a signature epitope distribution that is unique to each mAb
Good Antibody Specificity Was Achieved
0.1% New Epitope Exposures can be Quantified with the ELISA (QL=0.1%)

Spike Testing for the mAb Variable Region with InnoBridge ELISA
0.1% New Epitope Exposures Can Be Quantified with the ELISA (QL=0.1%)

Spike Testing for the mAb Constant Region with InnoBridge ELISA
For Quantitation Each mAb can Build a Standard Curve with QL = 0.1%

8 M urea denatured mAb was spiked into native mAb at 0, 0.1%, 0.2% and 0.5% respectively, conformational array ELISA was used to detect the spiked samples.
Sensitivity of the Antibody Array ELISA Typical Assay at 5 µg/ml.

Different Antibodies in the Array
mAb Conformational “Hot Spots”----Variable Region

Temperature Stability measured in InnoBridge ELISA

Absorbance at 450nm

LC-Fv-Fc

LC-CDR2

Ab1 Ab2 Ab3 Ab4 Ab5 Ab6 Ab7 Ab8 Ab9 Ab10 Ab11 Ab12

4C
37C
50C
60C
mAb Conformational “Hot Spots”----Constant Region

Temperature stability measured in InnoBridge ELISA

Next to Hinge Region

Absorbance at 450nm
3. Applications in Biosimilar mAb Conformational Analysis
Marketed Products with Antibody Arrays Available

<table>
<thead>
<tr>
<th>mAb Name</th>
<th>Trade Name</th>
<th>Composition</th>
<th>IgG Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bevacizumab</td>
<td>Avastin</td>
<td>Humanized mAb</td>
<td>IgG1</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>Erbitux</td>
<td>Humanized mAb</td>
<td>IgG1</td>
</tr>
<tr>
<td>Alemtuzumab</td>
<td>Campath</td>
<td>Humanized mAb</td>
<td>IgG1</td>
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<tr>
<td>Rituximab</td>
<td>Rituxan</td>
<td>Chimeric mAb</td>
<td>IgG1</td>
</tr>
<tr>
<td>Adalimumab</td>
<td>Humira</td>
<td>Human mAb</td>
<td>IgG1</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>Herceptin</td>
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<td>IgG1</td>
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<td>Synagis</td>
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<td>IgG1</td>
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<tr>
<td>Infliximab</td>
<td>Remicade</td>
<td>Chimeric mAb</td>
<td>IgG1</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>EPO</td>
<td>Human protein</td>
<td>Non-mAb</td>
</tr>
</tbody>
</table>
Summary of Outcomes of Biosimilar mAb Testing with Antibody Arrays from Multiple Customers

1. Outcome-1: Highly similar, no noticeable difference.
2. Outcome-2: Similar, with minor differences.
3. Outcome-3: Significantly different.
4. Some differences are correlated with changes in bioassay, Fc gamma binding, glycosylation patterns.
5. “Hot spots” observed from multiple customers on the same biosimilar molecule.
6. Innovator molecule also has conformational variation in certain regions.
7. Cell line and process causes are being investigated.
Structural Assignment for the region of difference: Ab9
Structural Assignment for the region of difference: Ab21
Variable Region Profile of Seven Marketed mAb Drugs
Even with >95% sequence identity, each of the mAb constant region has unique and stable surface epitope exposure pattern.
mAbs under Clinical Development: each mAb is unique in its 3-D structure (epitope exposure)

3 mAbs failed during clinical development (mAb3,4 and 7) seems to have more surface epitope exposure compared to the mAbs made it to the market.
Potential Applications for the Antibody Array Technology

- Cell Line Selection
- Process Development
- Formulation Development
- Comparability Studies
- Product Characterization
- Antibody-Drug Conjugates (ADCs)
4. Conclusions

- Antibody arrays were developed against 8 marketed monoclonal antibody drugs and one for novel mAbs.
- Each antibody array provides a unique signature for the mAb, reflecting its surface exposure and extent of exposure.
- The antibody array is sensitive, systematic and relatively high throughput.
- It correlates well with stability and bioassay data, **however more information can be acquired with the Antibody Array analysis that may not be detected by other technologies including bioassays.**
- It can be applied to many stages of biologics development, from cell line selection to product release.
- The technology can also be applied to novel mAb discovery and study antibody-drug conjugates (ADCs).