Quantification of Neutralizing Antibodies to Biopharmaceuticals using a Novel Cell-Based Assay Platform Technology

Michael G Tovey,
Director, Laboratory of Viral Oncology,
Institut André Lwoff,
Villejuif, France
tovey@vjf.cnrs.fr
Regulatory Perspective on Monitoring for Anti-Drug-Antibodies

- Monitoring should be clinically driven
- PK data must be analyzed in conjunction with immunogenicity assays
- Cell-based assays should be used to detect neutralizing antibodies *when ever possible*
- Inhibition of receptor binding does not *always* equate to neutralization of biological activity. Abs can neutralize biological activity without inhibiting receptor binding
iLITE™ Cell Based Assays for the Quantification of Biopharmaceuticals: Objectives

- Replace diverse complex biological endpoints with a single common endpoint
- Eliminate assay variation due to genetic & epigenetic changes associated with continuous cultivation of cells *in vitro*
- Translate Biopharmaceutical Industry Best Practice into the Development of Cell-Based Assays
Quantify Activity Without the Need for Live Cells or Cell-Culture
Cytokine Signal Transduction

Drug-Specific Regulatory Element

SV40 Min. prom.

Luciferase

SV40 Poly A

Intron Human β-globine
iLite™ Reporter-Gene Assays: Strategy

- Stable Transfectant
- Sub-clone
- Master cell bank
- Working cell bank
- Defined cell replication step
- Chemical Treatment
- Cryo-preservation
- Manufactured under ISO 13485 (cGMP)
*iLite*™ Reporter-Gene Assays

- Rapid and precise
- No cell culture
- Obviates assay variation associated with cell growth
- > 3 year stability – 80°C
- Fully automated HTS
- No specialized facilitates required
- Facilitates transfer to CRO
Neutralization Assay Performance: Clinical Implications

- Sensitivity of assay determines minimum Nab cutoff value
- Low Nab titer: false negative using insensitive assay
- *iLite* neutralization assay superior sensitivity to use of live cells
Quantification of Neutralizing Antibodies to TNF-α Antagonists
Detection of antibodies to TNF-α antagonists

- TNF-α signals through NFκB
- Numerous other cytokines: IL1-β, IL-2, IL-4, IL-10, IL-18, IFN β, etc.
  also employ the NFκB pathway
TNF alpha Antagonist
10 LU/ml

Dilutions

Serum samples
In serial Dilution

10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
10^{-8}

Controls:
Cells + Serum alone
Cells + Serum + TNF

TNF alpha
10 LU/ml

10’

10’

5 h

Reporter
Cells

BriteLite

Reading
iLite™: Detection of TNF-α in Sera from Patients with Rheumatoid Arthritis

- 0/112 Samples analyzed exhibited detectable levels of TNF-α
**iLite™** Quantification of Neutralizing antibodies to TNF-α Antagonists

- Robust assay no interference from circulating levels of TNF-α (like) activity
- Readily detect circulating levels of TNF-α antagonists (activity)
- Readily detect NAbs to TNF-α antagonists
- Readily distinguish between NAbs to different TNF-α antagonists
- A single assay for all TNF-α antagonists, allows direct comparisons of relative immunogenicity of drugs, including novel drugs in development or biosimilars
<table>
<thead>
<tr>
<th>Serum #</th>
<th>Patient #</th>
<th>TNF-alpha Antagonist</th>
<th>Current Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Etanercept</td>
<td>Adalimumab</td>
</tr>
<tr>
<td>1</td>
<td>0123247</td>
<td>np</td>
<td>np</td>
</tr>
<tr>
<td>2</td>
<td>0124063</td>
<td>np</td>
<td>np</td>
</tr>
<tr>
<td>3</td>
<td>0124064</td>
<td>np</td>
<td>np</td>
</tr>
<tr>
<td>4</td>
<td>0130022</td>
<td>np</td>
<td>np</td>
</tr>
<tr>
<td>5</td>
<td>0207179</td>
<td>np</td>
<td>np</td>
</tr>
<tr>
<td>6</td>
<td>0208007</td>
<td>np</td>
<td>np</td>
</tr>
<tr>
<td>7</td>
<td>0208014</td>
<td>np</td>
<td>np</td>
</tr>
</tbody>
</table>

Duration of treatment in months

- 4 months
- 12 months
- 18 months
- np
- 6 months
- 3 months
- None
iLite™ Quantification of Neutralizing antibodies to Anti-inflammatory Biologicals

- Simultaneous quantification of drug and anti-drug NAbs in the same assay
- A common assay format for TNF-α antagonists, anti-CD20 Mabs or other anti-inflammatory biologicals
- Readily distinguish between NAbs to different TNF-α antagonists
- A common assay read-out for TNF-α antagonists, anti-CD20 Mabs and other anti-inflammatory biologicals allows direct comparisons of relative immunogenicity of drugs.
iLite™ Quantification of anti-Drug NAbs:

Future Developments
One-Step \textit{iLite}™ Neutralization Assay
Cytokine 10LU/ml

Max 6 Serum samples In serial Dilution

Reporter Cells

Steady Glow

Autocrine Reporter Cells + Max 96 serum samples

Reading
FL1 / RL > FL2 / RL

- Renilla Luciferase (RL)
- Firefly Luciferase (FL)
- Nab
- Cytokine
- Cytokine receptor
**One-Step Nab Assay**

- Quantification of circulating drug levels
- No sample manipulation
- No drug standard curve required
- No control samples required
- High degree of precision results normalized relative to internal control; CV 4 to 8%
- Results independent of cell number
- Results independent of serum matrix effects
- Ideally suited to high through-put
- Reduced assay time
iLite™ Technology

- Fast, easy, reliable
- Excellent lot-to-lot, day-to-day repeatability/reproducibility
- 3 years of market experience (Biogen Idec, Merck-Serono, BMS, Schering-Plough and others)
- Issued US Patent (USPN 7,740,556) and several pending applications
- ISO13485 audited, certified manufacturing facility (Biomonitor Ltd, Galway Ireland).
## Current Products (Biomonitor A/S)

**USE**

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>iLite™alphabeta</strong></td>
<td>Detection and quantification of Type I human IFNs (RUO).</td>
</tr>
<tr>
<td><strong>iLite™antibeta</strong></td>
<td>Detection and quantification of NAbs in sera of MS patients being treated with IFN-beta (RUO).</td>
</tr>
<tr>
<td><strong>iLite™antialpha</strong></td>
<td>Detection and quantification of NAbs in sera of patients being treated with IFN-alpha (RUO).</td>
</tr>
<tr>
<td><strong>iLite™alphabeta CE</strong></td>
<td>CE Marked assay for the detection and quantification of IFN-alpha in patient sera to replace the CPE assay as an aid to the physician in optimizing therapy.</td>
</tr>
</tbody>
</table>
Products in Development (Biomonitor A/S)

**USE**

*iLite™ anti-infliximab CE*  
CE Marked assay for the detection and measurement of NAbs produced against commercial formulations of infliximab in patient sera.

*iLite™ anti-etencept CE*  
CE Marked assay for the detection and measurement of NAbs produced against commercial formulations of etanercept in patient sera.

*iLite™ anti-adalimumab CE*  
CE Marked assay for the detection and measurement of NAbs produced against commercial formulation of adalimumab in patient sera.