Characterization of Therapeutic Antibodies Derived from a Novel Yeast Expression Platform Using Bio-Layer Interferometry (BLI)

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Antibodies are successful therapeutics

• **Key Elements of Pharmaceutical Armamentarium**
  - Well accepted by both patients and physicians
  - Broad adoption by Pharma

• **Commercially Successful**
  Rituxan  ~$3.8 Bn/year
  Remicade  ~$3.5 Bn/year
  Herceptin  ~$2.9 Bn/year
  Avastin  ~$2.3 Bn/year
  Humira  ~$1.9 Bn/year
Three basic manufacturing problems facing the industry today

- **High Cost**
  The cost of manufacturing novel antibody therapeutics results in extremely high cost to patients.

- **Limited Capacity**
  The pharmaceutical industry was to spend $3.5 Bn in 2006 building new manufacturing capacity *just to meet current demand*.

- **Need for Accelerated Development Timelines**
  Still requires 3+ years from novel antibody discovery to first-dose in man
Alder is revolutionizing the way therapeutic antibodies are identified, selected, developed and produced.
ABS: Shortened timelines, better antibodies.

<table>
<thead>
<tr>
<th></th>
<th>ABS</th>
<th>Hybridoma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time</strong></td>
<td>2.5 months</td>
<td>18 months</td>
</tr>
<tr>
<td><strong>Diversity</strong></td>
<td>&gt;10 epitopes</td>
<td>2</td>
</tr>
<tr>
<td><strong>Function</strong></td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td><strong>Modifying MAbs</strong></td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td><strong>Affinity</strong></td>
<td>5 pM</td>
<td>5000 pM</td>
</tr>
</tbody>
</table>
BioLayer Interferometry (BLI): Our technology of choice for antibody characterization

- A layer of molecules attached to the tip of an optic fiber creates an interference pattern at the detector.
**BioLayer Interferometry (BLI):** Our technology of choice for antibody characterization

- A layer of molecules attached to the tip of an optic fiber creates an interference pattern at the detector.
- Any change in the number of molecules bound causes a measured shift in the pattern.

Source: Fortebio
**BioLayer Interferometry (BLI):** allows for label-free, real time, data collection

- Any change in the number of molecules bound causes a measured shift in the pattern

- Real-time measurements
- No background subtraction
Binning antibodies to multiple epitopes in real time using label-free antigen

Control Ab competes binding of AB4 to label-free antigen
Binning antibodies: up to 8 at the time!

Control Ab does not compete binding of AB1/2/3 to label-free antigen
“Antigen 1” signals via receptor on cell membrane

Antigen1 signaling steps: I) Hetero dimerization
II) Tertiary complex formation III) Activation
Mechanistic characterization of ALD antibodies

ABS derived antibodies cover novel epitopes and all mechanistic possibilities within “Antigen 1”
BLI Allows for multi-complex formation and MOA studies

- Biotinylated antigen
- Streptavidin sensor
- Tertiary complex
- Dissociation conditions
- Buffer
- Heterodimer

Diagram:

- Streptavidin sensor
- Tertiary complex
- Dissociation conditions
- Buffer
- Heterodimer
- Biotinylated antigen
BLI Allows for multi-complex formation and MOA studies

Streptavidin sensor

Biotinylated antigen

Tertiary complex

Dissociation conditions

Buffer

Heterodimer

Antibody blocks heterodimerization

Antibody binding
ABS derived antibodies block several steps of “Antigen 1” signaling complex

anti αFc sensor (pre-Protein A)

Tertiary complex
heterodimer
Antigen1
Control (non blocker) antibody
ABS derived antibodies block several steps of “Antigen 1” signaling complex

anti $\alpha$Fc sensor (pre-Protein A)

Tertiary complex

heterodimer

Antigen1

Control (non blocker) antibody

Antibody blocks tertiary complex formation

“Blocker antibody”
ABS produces high affinity antibodies

- BLI allows for quick kinetic determinations with easy to use software and simple reports.
Kinetic properties of ABS derived antibodies against “Antigen1”

<table>
<thead>
<tr>
<th>ALDER ANTIBODY</th>
<th>Kd pM</th>
<th>Ec50 pM</th>
<th>Epitope Bin</th>
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<tbody>
<tr>
<td>Antibody1</td>
<td>45</td>
<td>53</td>
<td>IV</td>
</tr>
<tr>
<td>Antibody2</td>
<td>16</td>
<td>198</td>
<td>I</td>
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<tr>
<td>Antibody3</td>
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<td>Antibody10</td>
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<td>IV</td>
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Mab X-press highlights

• Yeast Pichia pastoris: well established protein expression system.
  – Pichia products therapeutic proteins have extensive history in man
  – System able to produce gm/L quantities of protein
  – Provides for accelerated development timelines
    • Rapid fermentation
    • Simplified product purification
    • Expedited cell banking
    • No Viral testing
  – Utilizes existing microbial mfg capacity for large-scale production
  – **Overall: stable supply for chronic disease indications.**
• Alder proprietary IP for antibody expression
Titer generation using Mab X-press

Need: Real time measurement of titer development
Antibody titer determination with BLI

- Octet Binding Curve = rate of increase in optical thickness as the sample binds to the sensor.
- Different proteins concentrations result in different binding curves

Source: Fortebio website
In-house standard curve generation using the octet

Dynamic range: 1 to 200ug/mL
## Validation of Octet data

<table>
<thead>
<tr>
<th></th>
<th>“Run1” ProtA (mg/L)</th>
<th>“Run1” Octet (mg/L)</th>
<th>“Run2” ProtA (mg/L)</th>
<th>“Run2” Octet (mg/L)</th>
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<tr>
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Octet versus protein A
Summary

• ABS is a rapid monoclonal antibody discovery platform
  – Comparable timing to phage but better quality
  – No need for affinity maturation
  – BLI allows for rapid kinetic analysis of multiple antibodies

• Superior epitope coverage to hybridoma
  – Antibody binning using BLI can be done with either biotinylated antigen or biotinylated antibody
  – Multiple coverage of epitopes within an antigen allow for novel mechanisms for therapeutic interventions. BLI enables fast and real-time mechanism of action analysis.

• Mab X-press lowers antibody production costs and shortens time lines.
  – BLI supports fast titer determination of Mab X-press antibodies during production.
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