Efficient production of recombinant proteins in the aquatic plant *Lemna*  
Kevin Cox
Biolex corporate profile

LEX System℠

- Recombinant protein expression system which facilitates expression of hard-to-make proteins, optimized monoclonal antibodies and antigens
- Active GMP manufacturing facility

Financing

- Venture capital backed biotherapeutics company

Partner Proteins

- Lead product, Locteron™, in Phase 2
The LEX System: Ideal for expression of therapeutic proteins

- Based on aquatic higher plant, *Lemna*
- CHO-like features:
  - Clonal
  - Doubles every 36 hours
  - High protein content
  - Complex post-translational processing
  - Secretes recombinant protein
- Contained and controlled cGMP facilities
LEX System
Other key attributes

• Fast Development / Scale Up
  – Commercial line in 6 months
  – IND in 18 months
  – Launch facility in place in 2 to 3 years

• Delivers Superior Economics
  – High expression levels, scalable, simple
  – No animal-derived components
  – GMP facilities with low operating and capital costs
  – Freedom to operate
  – Avoidance of mammalian cell royalties
Biolex Large-Scale Production Facilities Offer Significant Benefits for Antibody Production

<table>
<thead>
<tr>
<th>Faster Scale-Up (Design/Build/Validate)</th>
<th>LEX System</th>
<th>Mammalian (CHO) system</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0</strong> to <strong>3 Years</strong></td>
<td>LEX System</td>
<td>Mammalian (CHO) system</td>
</tr>
<tr>
<td><strong>Lower CAPEX</strong></td>
<td>LEX</td>
<td>Mammalian (CHO) system</td>
</tr>
<tr>
<td><strong>$0</strong> to <strong>$200 Million</strong></td>
<td>LEX</td>
<td>Mammalian (CHO) system</td>
</tr>
<tr>
<td><strong>Lower COGS</strong></td>
<td>LEX System</td>
<td>Mammalian (CHO) system</td>
</tr>
<tr>
<td><strong>$0</strong> to <strong>$100 / Gram</strong></td>
<td>LEX System</td>
<td>Mammalian (CHO) system</td>
</tr>
<tr>
<td><strong>$0</strong> to <strong>$200 / Gram</strong></td>
<td>LEX System</td>
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</table>

Note: Based on detailed LEX System design and cost model for a mAb at 200 kg/year output and Industry benchmarks for a similarly sized CHO facility
The process of *Lemna* transformation and line screening

Transgenic Line Screening:
- Analyze expression
- Select line for scale-up – 6 months from amino acid sequence to production line
Rapid screening of hundreds of plant lines in CLC process

mAb Total Soluble Protein (TSP)

% TSP

Superior Economic Level

7%

Transgenic Lines
Recombinant proteins successfully expressed in the LEX System

Molecular Weight (kD)

1  10  100  1,000

Monomers

Dimers

Tetramers

Superior economics than other systems

Hard to make in other systems

Optimized Glycosylation

Confidential
LEX System demonstrated to be broadly applicable to mAbs

- Expression and purification of 12 antibodies to high purity
  - Multiple isotypes (IgG1, IgG2, IgG4)
  - Glycosylated and aglycosylated forms
- Comparable to CHO-derived reference mAbs in all cases
Immunolocalization of mAb in *Lemna*

* mAb is localized in the apoplast and expressed throughout the plant
Lemna derived mAbs are comparable to mammalian cell derived reference standard

- **Tests**
  - Thermal stability
  - IEF
  - SDS-PAGE
  - Western blot
  - N-terminal sequence
  - Chemical stability
  - Aggregation propensities
  - HPLC
  - Bioactivity
  - Potency (cell-based assay)
  - Tryptic digest map
  - Carbohydrate analysis

<table>
<thead>
<tr>
<th>Antibody</th>
<th>$Tm_1(°C)$</th>
<th>$Tm_2(°C)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEX mAb</td>
<td>75</td>
<td>83</td>
</tr>
<tr>
<td>CHO mAb</td>
<td>73</td>
<td>83</td>
</tr>
</tbody>
</table>

Typical SEC-HPLC Profile

- > 99% Monomer
- Comparable to Mammalian Ref. Standard
Comparison of plant and mammalian N-glycan structures on recombinant mAbs

Generalized plant glycan

Generalized mammalian glycan

N-acetyl glucosamine
mannose
B1,4 galactose
α1,3 fucose
α1,6 fucose
β1,2 xylose
sialic acid
protein backbone
Glycan optimization of mAbs

- *Lemna* Wild-type glycosylation is more homogeneous and provides ideal backbone for glycan optimization

- RNAi based approach used to eliminate plant-specific carbohydrates
Binary vector for co-expression of mAb and RNAi cassette
Analysis of top mAb expressing lines for Fuct / Xylt activity
Analysis of labeled mAb N-glycans

- Biolex Optimized N-glycans – significant in 2 ways
  - Single human-like species provides homogeneity
  - Complete elimination of plant-specific sugars
Consistency of N-glycans among different plant lines and scales - MALDI-TOF Analysis

WT

Line 20

Line 52

Line 225
The LEX System for production of recombinant vaccines

- The *Lemna* system is an ideal platform for production of recombinant vaccines
- Biolex has an ongoing collaboration with Merial
  - Biolex has demonstrated successful expression of 3 viral antigens in the LEX System
  - *In vivo* studies in planning
- PoC
  - Lemnaceae derived plant extracts expressing VP2 antigen were shown to provide protection against IBDV in chicks
  - Resulted in superior immune response compared to conventional whole virus vaccine
    - Z. Penzes, 2007 Jun 18-20, PBVA, Verona
Scale Vessel (SV): Bioproduction format

- Scale Vessel
  - 1st level of scale (300X research scale)
  - Aseptic operation
  - Suitable for 100s of grams to 10s of kilograms
  - Currently being used in production of phase 1 and 2 clinical material
Large Bag: Bioproduction format

• Large Bags
  – 2\textsuperscript{nd} level of scale (6000X research scale)
  – Easily stacked
  – Inoculate and harvest in place with aseptic connections
  – Disposable, no cleaning validation
## Consistency Across Scale

<table>
<thead>
<tr>
<th>Scale (g biomass)</th>
<th>% TSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV (1g)</td>
<td>6.7</td>
</tr>
<tr>
<td>SV (300g)</td>
<td>6.8</td>
</tr>
<tr>
<td>Bag (6000g)</td>
<td>6.5</td>
</tr>
</tbody>
</table>

~ 1g / kg biomass
Comparison of $N$-glycan homogeneity from research to commercial scale

Research Scale
1 g

Pilot Scale
300 g

Commercial Scale
6,500 g
Biolex has “Industrialized” the LEX System and has Established GMP Capabilities

• Contained, controlled 15,000 ft² GMP facility in place
  – ca. 2.5kg mAb capacity
• Non-classified upstream area
  – Growth and harvest of plant material
  – Scale vessels used for growth of plant material
• Classified downstream area
  – Post harvest recovery and purification
Kit Creek Pilot / Launch Production Facility

- 40,000 square foot facility will be operational in 2008
- To be used for phase 3 clinical production and launch of Locterone product
- Large bag technology to be implemented for growth of plant material
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