APPROACHES TO IMPROVING THE PERFORMANCE OF MAMMALIAN CELL CULTURES FOR PROTEIN PRODUCTION

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The Challenge of the MAb Market

- Global market for Monoclonal Antibody Therapeutics reached a total of $7.2 billion in 2003
- Compound average annual growth rate of 53% over previous 5 years
- More than 370 recombinant antibody product are currently in the pipeline
- Currently fifteen rMAbs on the market with many more in development
- Several are ‘blockbuster’ therapeutics
- High dose requirement – 10s to 100s Kg per annum required
- Challenge: produce large quantities with cost and time efficiency

Marketresearch.com 2004
Research and Markets 2004
Meeting the Challenge

- Can large quantities simply be obtained by scaling up and up?

- Cell lines
  - Highly productive
  - Stable

- Cell culture processes
  - Robust
  - Scalable
20,000 Litre Large Scale Reactor
Meeting the Challenge

- Can large quantities simply be obtained by scaling up and up?

- Cell lines
  - Highly productive
  - Stable

- Cell culture processes
  - Robust
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The Benefits of Increasing Productivity

The diagram illustrates the relationship between product titre (g/L) and the number of batches required, as well as the relative cost, for two scales: 2000L and 5000L. The graph shows that as the product titre increases, the number of batches required decreases significantly, leading to a reduction in relative cost.
Highly Productive Cell Lines

- Host cell
- Expression system
- Transfection and selection protocol
- Rapid creation

- Goal to create stable, high producing cell lines
  - Grow in suspension culture
  - Grow in a chemically defined, animal component-free media
- Optimise culture
  - High specific productivity, high cell numbers which can be maintained for extended time
**Highly Productive Cell Lines II**

- **Host cell**: CHOK1SV
- **Expression system**: GS
- **Electroporation and selection protocol**: MSX
- **Rapid creation**: 20 weeks

- **Early phase clinical supply (Uncloned)**
  - cDNA to cGMP in a generic process in <12 months

- **Late phase clinical supply (Clonal)**
  - Marry cell line with optimised process
Host Cell and Expression System

- Developed CHOK1SV (suspension variant)
  - Grows as single cell suspension
  - Pre-adapted to growth in chemically defined, animal component-free media
  - Exhibits good growth characteristics
    - Reach high maximum viable cell concentration
    - Able to maintain cultures at high cell viability

- The GS Gene Expression System
Timeline Reduction with CHOK1SV

- Use of suspension variant of CHO K1 pre-adapted to growth in chemically defined, animal component-free (CDACF) media substantially reduces time taken to generate cell lines

**Serum-Containing Process**

- Vector construction
- Transfection
- Selection and Expansion
- Adaptation to Suspension
- Growth

**CDACF Process**

- Vector construction
- Transfection
- Selection and Expansion
- Adaptation to Suspension
- Growth

20 weeks
Host Cell and Expression System

- Developed CHOK1SV (suspension variant)

- The GS Gene Expression System
  - Glutamine synthetase (GS) used as a selectable marker
  - Glutamine omitted from culture media as selective pressure
  - Further selection pressure applied with methionine sulphoxamine (MSX) - a specific inhibitor of GS

- Focus on recent developments creating GS-CHO cell lines
Host Cell and Expression System

\[
\text{ATP} \quad \text{ADP} + \text{Pi} \\
\text{NH}_4^+ + \text{glutamate} \quad \rightarrow \quad \text{glutamine} \\
\text{MSX}
\]
Host Cell and Expression System

- Developed CHOK1SV (suspension variant)

- The GS Gene Expression System
  - Glutamine synthetase (GS) used as a selectable marker
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- Focus on recent developments creating GS-CHO cell lines
GS Expression Vectors

- Antibody genes driven by strong promoters
- GS gene driven by weak promoter
- Biases for selection of rare integration into transcriptionally active sites in genome
- Both light chain and heavy chain on one vector
- Range of constant region vectors available preformatted to facilitate antibody cloning
Finding High Producers

- High producers are infrequent

- Probability distribution of antibody productivities for primary GS-CHO transfectants (24 well plates)
  - 90% transfectants produce less than 90 mg/L
  - 1.5% transfectants produce more than 150 mg/L
Cell Line Screening

- Highly productive transfectants are rare even with a good selection system
- How can the hit rate for finding highly productive cell lines be increased?

- Various approaches to improve screening process
  - Increase number of colonies created
  - Improve stringency of selectable marker to eliminate low producers
  - High throughput methods (FACS + cell surface product capture)
  - Early screening will not necessarily indicate growth characteristics in manufacturing process
Influence of electroporation conditions for GS-CHO cell lines with cB72.3 antibody

<table>
<thead>
<tr>
<th>Electroporation condition</th>
<th>Numbers of stable transfectants</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68</td>
</tr>
<tr>
<td>2</td>
<td>124</td>
</tr>
<tr>
<td>3</td>
<td>197</td>
</tr>
</tbody>
</table>

2.5 x 10^6 cells electroporated
Influence of selection conditions for GS-CHO cell lines with cB72.3 antibody

Selection conditions - MSX concentration

Cell lines have not been amplified.
Optimising Transfection and Selection III

- Evaluation of conditions and MSX concentrations during electroporation

<table>
<thead>
<tr>
<th>Electroporation condition</th>
<th>MSX (µM)</th>
<th>Stable transfectant numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>32</td>
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<td>2</td>
<td>25</td>
<td>124</td>
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<tr>
<td></td>
<td>50</td>
<td>57</td>
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<td>3</td>
<td>25</td>
<td>197</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>70</td>
</tr>
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</table>
Improving GS Vectors for Antibodies

- Free light chain often seen in cultures
- Does first gene (LC) interfere with expression of second gene (HC)?
- Can levels of LC and HC be balanced?
  - Transcription blocker
- Must the LC gene be first?
  - Reverse the order and put HC first
- Can stronger promoters be used?
Evaluating Improvements to GS Vectors

**STATIC CULTURE**

1. Transfect host cells with vector
2. **3 - 4 weeks**
3. **100 transfecteds**
4. **Identify single colonies per well**
5. **Transfer to 24 well plate**
6. **2 weeks**
7. **100 transfecteds**
8. **productivity assessment (quantitative)**
9. **100 data points**
10. **Compare against control**
Improving GS Vectors for Antibodies

- Control Vector (LC-HC)
- Reverse Orientation (HC-LC)
- Transcription Blocker (LC-TB-HC)
- Stronger Promoter (LC-HC)

Antibody (mg/L)

n=100
Improving GS Vectors for Antibodies

- Order of LC and HC is important even in CHO cells
  - LC preferred upstream

- Presence of a transcription blocker provides no benefit

- Promoter strength appears to be finely balanced
Improving GS Vectors for Antibodies

- Control Vector (LC-HC)
- Reverse Orientation (HC-LC)
- Transcription Blocker (LC-TB-HC)
- Stronger Promoter (LC-HC)

Antibody (mg/L)

n=100
Affinity-matrix surface capture (AMSC)

- Fluorochrome-labelled detection antibody
- Secreted antibody
- Biotinylated Protein A
- Neutravidin bridge
- Biotinylated cell surface
Flow cytometric analysis

AMSC-labelled GS-CHO cells

*Negative control*

*Positive control*

*LB01*

*CHOK1 SV transfectant pool (pcB72.3)*
Finding High Producers I

Transfect host cells with vector

### STATIC CULTURE

3 - 6 week

- 200-300 transfectants
  - Productivity assessment (quantitative)

1 week

- 60-100 transfectants
  - Preliminary productivity assessment (quantitative)

4 weeks

- 30-60 transfectants
  - Adapt to protein-free culture

### SUSPENSION

(shake flask culture)

8 weeks

- 30-60 transfectants
  - Preliminary productivity assessment (quantitative)

3 weeks

- 5-10 transfectants
  - Fed-batch assessment of growth and productivity

Select 3 cell lines for further analysis
Finding High Producers II

Shake-flask cultures operated in fed-batch mode

![Bar chart showing harvest product concentration (mg/L) for different candidate cell lines.](image-url)
Finding High Producers II

Shake-flask cultures operated in fed-batch mode

Harvest Product Concentration (mg/L)

Candidate Cell Lines
## Finding High Producers III

<table>
<thead>
<tr>
<th>Bioreactor</th>
<th>Maximum Viable Cell Concentration ($10^6$ cells/mL)</th>
<th>Product Concentration (g/L)</th>
<th>Specific Production Rate (pg/cell/h)</th>
<th>Harvest day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory-scale</td>
<td>9.42</td>
<td>1.6</td>
<td>0.78</td>
<td>15</td>
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<tr>
<td>(10 L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pilot-scale</td>
<td>10.78</td>
<td>1.9</td>
<td>0.76</td>
<td>17</td>
</tr>
<tr>
<td>(130 L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manufacturing</td>
<td>9.66</td>
<td>1.4</td>
<td>0.78</td>
<td>15</td>
</tr>
<tr>
<td>(200 L)</td>
<td></td>
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</table>
Highly Productive Cell Culture Processes

- Significant potential to improve processes
  - Medium design and feeding strategies
  - Physicochemical environment
  - Focus on developing generic platform processes
- Iterative process
  - Monitor nutrient usage and ensure that extra of those which are depleted is added back next time
  - Developed improved basal media
  - Two continuous feeds
- Waste metabolites
  - Control lactate accumulation with pH
  - Ammonia is a substrate for the GS enzyme so accumulation is not usually an issue
Animal Component-Free Processes

- Why animal component-free?
  - Increasing emphasis from regulatory authorities on removal of animal-derived raw materials from antibody production processes
    - Potential source of adventitious agents and product contaminants
  - Sourcing of animal derived proteins, such as New Zealand bovine serum albumin, becoming more difficult as demand increases due to expanding worldwide production capacity
    - Potentially a similar issue with pharmaceutical grade components used as protein replacements
Full Chemical Definition

- Knowledge of media components gives full control of process optimisation
- Lot to lot variability of raw materials reduces manufacturing process robustness and consistency
  - Issue associated with both hydrolysates and peptones
  - May necessitate extensive raw material testing to identify ‘good’ raw material lots
- Simplification of downstream processing
  - Reduced protein contaminant levels
  - Increased harvest material purity
Process Improvement I

Improving Cell Growth

![Graph showing cell growth over time with iterations 1 and 2 with different conditions.](Image)

- **Iteration 1, 22H11**
- **Iteration 2, 22H11**
- **Iteration 2, LB01**
Process Improvement II

Improving Cell Growth

Viable Cell Concentration (10⁶/mL)

Time (h)

It. 2  It. 3  It. 4  It. 5  Cl. CY01
Process duration

- 22H11 orig
- 22H11 v1
- 22H11 v2
- LB01 v2
- LB01 v3
- LB01 v4
- LB01 v5
- CY01 v5
Specific productivity

- 22H11 orig
- 22H11 v1
- 22H11 v2
- LB01 v2
- LB01 v3
- LB01 v4
- LB01 v5
- CY01 v5
Process Improvement

Product Concentration (mg/L) vs. Time Integral of Viable Cell Concentration (10⁹ cell h/L)

- It. 2
- It. 3
- It. 4
- It. 5
- Cl. CY01
Product concentration

![Product concentration chart](chart.png)
# CHO Process Optimisation Summary

<table>
<thead>
<tr>
<th>Process</th>
<th>Antibody (mg/L)</th>
<th>Fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original cell line</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>Iteration 1</td>
<td>334</td>
<td>2</td>
</tr>
<tr>
<td>Iteration 2</td>
<td>585</td>
<td>4</td>
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<tr>
<td>New cell line</td>
<td>1917</td>
<td>14</td>
</tr>
<tr>
<td>Iteration 3</td>
<td>2829</td>
<td>20</td>
</tr>
<tr>
<td>Iteration 4</td>
<td>3560</td>
<td>26</td>
</tr>
<tr>
<td>Iteration 5</td>
<td>4301</td>
<td>31</td>
</tr>
<tr>
<td>Cloned cell line in Iteration 5</td>
<td>5520</td>
<td>40</td>
</tr>
</tbody>
</table>
Summary

- Creation of highly productive cell lines is the sum of many parts
  - Host cell and expression system
  - Transfection and selection
  - Strategies to identify the highest expressers

- Significant potential for further yield enhancement
  - Process optimisation
  - Lonza’s philosophy of generic platform development based on chemical definition and animal component-free processes
Acknowledgements

- Development – Lonza Biologics Slough, UK
  - Cell Culture Process Development
  - Assay Development
  - Process Scale Up and Support

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