Challenges and Opportunities in the Large Scale Production of Therapeutic Proteins

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Significance of Mammalian Cell Processes

- Commercial significance of biopharmaceutical proteins is driving progress in process development
- Approx. 40 products made in mammalian cell culture, about half of which are antibodies

- CHO 27 (10 Mabs)
- NS0, SP2/0, hybridomas etc 8 (8 Mabs)
- Other 4

- Several hundred more proteins in development
Key Issues in Manufacturing

- Cost efficient manufacturing process – especially for high volume products
  - High dose requirements, particularly for antibodies, leads to large volume demand (10’s to 100’s kg/year)
  - Estimated 2004 protein demand > 2000kg (mostly Mabs and fusion proteins) (UBS)
- Speed in development, especially for early phase clinical material, where process development and provision of first GMP material is likely to be on critical path
- Control of product properties e.g. glycosylation
Cost Efficient Manufacturing Process

- Demand for large volume products has been a driver for:
  - Increased reactor size – up to 20000 litres (to realise economy of scale) 
  - Improved technology to increase cost efficiency both upstream and, with increasing titres, downstream
20,000L Bioreactor & Add Tanks
Portsmouth, New Hampshire
Purification 2.0M and 1.4M Columns
Technology Improvements for Upstream Technology

- Creation of highly productive cell lines
  - Efficient gene vectors, transfection methods, screening technologies
  - Improved host cell lines; variants (e.g. CHOK1 suspension variant) and engineered cell lines
  - Strategies for cell line improvement will increasingly be informed by better understanding of cell biology using modern „omics“ approaches
  - Cell line improvement also relevant to improving product characteristics

- Optimisation of fermentation process
  - Design of media and feeding strategies
  - Optimisation of physicochemical conditions

- For antibodies, titres of >1g/l now normal and 10g/l probably achievable
Optimisation of a GS-CHO Process

- Culture conditions for a GS-CHO making cB72.3 antibody were optimised
- Suspension variant of CHO-K1 isolated:
  - grows in chemically defined medium without need for adaptation (can take several months)
- Efficiency and stringency of transfection conditions increased to improve selection of highly productive clones
- Growth conditions further optimized
- More productive clone isolated
## Process Optimisation for a GS-CHO cell Line

<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>
</tr>
<tr>
<td>New cell line (CHOK1SV)</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>New Clone</td>
<td>5520</td>
<td>40</td>
</tr>
</tbody>
</table>
GS-CHO optimisation; productivity

[Charts showing antibody production and specific production rate across different process development stages.]
GS-CHO optimisation; growth parameters

![Graphs showing growth parameters for different stages and variants of GS-CHO.](slide12.png)
# Process Optimisation for a Model GS-NS0

## Chemically Defined, Protein-Free Bioreactor Process

<table>
<thead>
<tr>
<th>Process</th>
<th>Cumulative cell time (10⁹ cell·h/L)</th>
<th>cB72.3 antibody (mg/L)</th>
<th>Process duration (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum-free</td>
<td>640</td>
<td>476</td>
<td>12</td>
</tr>
<tr>
<td>Original protein-free</td>
<td>772</td>
<td>293</td>
<td>12</td>
</tr>
<tr>
<td>Iteration 1</td>
<td>1026</td>
<td>589</td>
<td>15</td>
</tr>
<tr>
<td>Iteration 2</td>
<td>1239</td>
<td>807</td>
<td>16</td>
</tr>
<tr>
<td>Iteration 3</td>
<td>1427</td>
<td>1035</td>
<td>16</td>
</tr>
<tr>
<td>Iteration 4</td>
<td>1405</td>
<td>1422</td>
<td>18</td>
</tr>
</tbody>
</table>
Key Issues in Manufacturing

- Cost efficient manufacturing process
- Speed in development
Speed in Development

- Predictive scale up systems – challenge is to predict manufacturing behaviour of cell lines at very early stage and select clones with e.g. appropriate growth characteristics

- Use of platform technology – culture media, feeds, growth conditions etc

- Design manufacturability into product molecule
Clonal Variation – GS-NS0 (non-optimised culture)

<table>
<thead>
<tr>
<th>Specific prod. rate (pg/cell/day)</th>
<th>Max. viable cell concn. (10^5 /ml)</th>
<th>Cumulative cell time (10^9 cell·h/L)</th>
<th>Product concn. (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>17</td>
<td>379</td>
<td>260</td>
</tr>
<tr>
<td>19</td>
<td>8</td>
<td>209</td>
<td>165</td>
</tr>
<tr>
<td>19</td>
<td>19</td>
<td>212</td>
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<td>24</td>
<td>22</td>
<td>518</td>
<td>535</td>
</tr>
<tr>
<td>31</td>
<td>19</td>
<td>573</td>
<td>750</td>
</tr>
<tr>
<td>60</td>
<td>14</td>
<td>320</td>
<td>790</td>
</tr>
</tbody>
</table>
Prediction of bioreactor behaviour from shake-flask model

![Graph showing value in reactor relative to shake-flask for antibody and Qp parameters](image)
Cell line creation often the slowest step – need to complete as rapidly as possible, ideally without compromising productivity or clonality criteria.
Traditional Development Process (Hypothetical)

- Cell Line Development
- Purification development & evaluation of virus reduction
- Cell bank preparation & testing
- Fermentation process development
- Analytical development
- cGMP manufacture

Year 1

Year 2
Approximate Timelines for Constructing and Selecting High-yielding Clonal Cell Lines

Traditional

- Transfection & selection
- Amplification & selection (1 round)
- Suspension evaluation (low serum)
- Limiting diln. cloning (2 rounds)
- Suspension evaluation (serum-free)
- Select lead cell lines

Non-amplified (e.g. GS-CHO)

- Transfection & selection
- Suspension evaluation (protein-free)
- Capillary-aided cell cloning (1 round)
- Suspension evaluation (protein-free)
- Select lead cell lines

Weeks
Speed in Development

- Cell line creation – how can timelines for cell line creation be reduced?
  - Avoid amplification
  - Avoid multiple cloning steps
  - Use of host cells for genetic manipulation that have been preselected to grow under manufacturing conditions (e.g. suspension culture in chemically defined medium)
  - Use transient expression or non-clonal cell line for early development studies
Approximate Timelines for Constructing and Selecting High-yielding Clonal Cell Lines

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~10 wks for 1 round

Non-amplified (e.g. GS-CHO)
- Transfection & selection
- Suspension evaluation (protein-free)
- Capillary-aided cell cloning (1 round)
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Weeks
Eliminating the need for amplification

- High stringency selection for integration events in highly efficient sites
- High throughput screening for (rare) highly productive variants
- Targeting of expression vector to transcriptionally highly active sites
- Vectors with elements that create genomic environment for high transcriptional activity
Glutamine synthetase (GS) gene expression system

- Expression vector encoding product gene plus GS gene, allowing glutamine synthesis
- GS is inhibited by methionine sulfoximine (MSX)
- Selection in glutamine-free medium for GS minus cell types (e.g. NS0)
- Selection in the presence of MSX for GS positive cell types (e.g. CHO)
- Only cells with GS gene (and hence product gene) survive
- Increase selection stringency - use weak promoter on GS gene - selects for rare integration into transcriptionally efficient sites in genome
- Expression of linked product gene, driven by strong promoter, enhanced by favourable integration site
Speed in Development

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- Select lead cell lines

~28 wks for 2 rounds
~9 wks
Cloning of Cell Lines

- Limiting dilution methods often used and require time consuming multiple rounds of cloning to provide high probability of clonality
- Single step method developed – capillary aided cell cloning
- Based on method described by Clarke and Spier (1980) Archives of Virology 63, 1-9
- Small droplets (1 microlitre) of culture drawn into capillary and deposited in microwell plates
- Visual confirmation of drops containing single cells
- Statistical analysis to establish probability of monoclonality
Speed in Development

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Non-amplified (e.g. GS-CHO)

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- Capillary-aided cell cloning (1 round)
- Suspension evaluation (protein-free)
- Select lead cell lines

~35 wks

~9 wks
**CHO suspension variant**

- Isolated variant of CHOK1 (CHOK1SV) capable of growth in suspension in chemically defined medium without need for adaptation period

- Used as host with GS expression technology

- High producing cell lines created (grams / litre)
Growth comparison: “old” vs. “new” GS-CHO cell lines growing in chemically defined medium
Speed in Development

- Cell line creation – how can timelines for cell line creation be reduced?
  - Avoid amplification
  - Avoid multiple cloning steps
  - Use of host cells for genetic manipulation that have been preselected to grow under manufacturing conditions (e.g., suspension culture in chemically defined medium)
- How can timelines be further reduced
Possible Solutions

How can programme length be shortened?

Duration can be reduced by moving straight from the transfection stage to the cloning stage, removing intermediate screening stage.
Reduction of screening timeline

- The pool of cell lines can be rapidly enriched for those with desired characteristics prior to cloning
  - Glutamine Synthetase (GS) Expression System indirectly enriches for high producers at transfection stage by use of the GS inhibitor MSX
  - Use second, rapid, method (FACS) to enrich directly for high producers between transfection and cloning stages
Cell Line Selection

Influence of Selection Conditions for GS-CHO Cell Lines with cB72.3 Antibody

Cell lines have not been amplified.
Affinity-matrix surface capture

- neutravidin bridge
- biotinylated Protein A
- fluorochrome-labelled detection antibody
- secreted antibody
- biotinylated-cell surface

EP1415158 A, Lonza
Analysis of AMSC-labelled GS-CHO cells producing a recombinant antibody

- Fluorescent signals for antibody-producing GS-CHO cells were substantially higher than for non-producing cells.
The questions:

- Does high fluorescence correlate with high productivity?

- Do cell lines generated by both the AMSC and conventional methods produce similar antibody concentrations in a model of the production process?
FACS enrichment

- Cell line construction and selection methods were run in parallel using a conventional protocol and a flow cytometric protocol

- Cell line CHOK1SV transfected with GS expression vector encoding HC and LC genes for a human IgG

- Cytometric method enriched cell populations for high producers

- For key selection stage, evaluation in fed-batch suspension culture, mean antibody concentration and mean Qp were the same
Conventional vs. AMSC-cloning: fed-batch, suspension culture
Impact of using AMSC-cloning approach

- Both methods generated a panel of cell lines having similar productivities.
- Enrichment of transfectant pool before cloning has shortened screening step by 9 weeks and potential for further improvement if FACS is qualified for single cell sorting.
Summary

- Very significant progress has been made in two key aspects of upstream mammalian cell process development
  - Productivity – titres of several grams per litre now seen, resulting from efficient expression technology based on GS and optimisation of fermentation process
  - Reduced timelines in cell line development
  - Significant opportunities for further improvement in many areas including productivity (cells and process) and speed
  - Improved understanding of biology underpinning productivity will increasingly inform process and cell design
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