Therapeutic antibodies – the Challenge

- High value market
  - Biopharma sales ca. $22bn in 2001: mammalian cell products represent ca. 60%
  - MAb market has grown from 1% of biopharma in 1995 to 14% in 2001
    - Polastro & Tulcinsky, SCRIP magazine Sep 2002.

- Fifteen licensed rMabs and large number in development

- High dose requirement leads to large volume demand (10’s to 100’s kg/year)

- Challenge: produce large quantities with cost and time efficiency
Industry drivers

- Capacity availability
  - Demand for large number of proteins (hundreds) in development
  - Material supply, up to 100s kg/year

- Cheaper
  - Improved yields of USP and DSP platform processes
  - Process optimisation for Ph III / in-market supply
Industry drivers

- Faster entry into clinic and market
  - Reduced USP and DSP development times through use of generic processes to supply PhI / II trials
  - Robust processes minimising risk of failure
  - Streamline regulatory aspects of processes

- Regulatory compliance
Mammalian cell culture: Expected capacity Increases

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>In-House</td>
<td>650,000</td>
<td>810,000</td>
<td>1,460,000</td>
</tr>
<tr>
<td>Contract Manufacturing Organisations (CMO)</td>
<td>190,000</td>
<td>320,000</td>
<td>510,000</td>
</tr>
<tr>
<td>Total Industry</td>
<td>840,000</td>
<td>1,130,000</td>
<td>1,970,000</td>
</tr>
<tr>
<td>% CMO</td>
<td>23%</td>
<td>28%</td>
<td>26%</td>
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A high yielding antibody manufacturing process is the result of:

- Selecting highly productive cell lines
  - Efficient gene expression and stringent selection
- Cell culture process supporting high viable cell concentration
  - Optimised process
- Minimising losses in primary recovery and purification
  - Optimised process
High level gene expression

- Strong promoter to drive expression of product gene(s)
  - Viral, elongation factor

- Increased copy number of product gene(s) that give proportional increase in gene expression
  - Co-amplification of product and selectable marker genes (e.g. DHFR) in presence of cytotoxic drugs (e.g. methotrexate)
  - Lower cell line stability compared to un-amplified cell lines

- Vectors with elements (e.g. SAR/MAR) that create genomic environment for high transcriptional activity

- Targeting of expression vector to genomic hot spot by homologous recombination
Improving the host cell line

- Cell line engineering
  - Glutamine independence using GS reduces ammonium accumulation
    - High ammonium levels reduce sialylation
  - Over-expression of anti-apoptosis genes
  - Maintain high viable cell concentrations for extended periods
  - Cell cycle genes

- Variant Selection
  - Cholesterol independent NS0 variant
  - Suspension variant of CHO
Cell line selection

- By definition, the transfectants with potentially the highest specific productivities are rare

- To find these rare events, it is necessary to have:
  - A transfection method that generates large numbers of stable transfectants
    - Maximise the range of productivities
  - Stringent selection to eliminate lower producers
  - High throughput methods e.g. FACS + cell surface product capture
# Cell line selection

Transfection and selection conditions for GS-CHO cell lines expressing cB72.3 antibody

<table>
<thead>
<tr>
<th>Electroporation condition</th>
<th>Selection condition MSX (µM)</th>
<th>Numbers of stable transfectants</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>57</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>197</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>70</td>
</tr>
</tbody>
</table>
Cell line selection

Influence of selection conditions for GS-CHO cell lines with cB72.3 antibody

Cell lines have not been amplified.
Cell line selection

Antibody production by non-amplified GS-CHO cell lines in a shake-flask model of a fed-batch production process

<table>
<thead>
<tr>
<th>Cell line ID</th>
<th>cB72.3 antibody concentration at harvest (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6</td>
<td>422</td>
</tr>
<tr>
<td>C7</td>
<td>514</td>
</tr>
<tr>
<td>C11</td>
<td>641</td>
</tr>
<tr>
<td>C12</td>
<td>632</td>
</tr>
<tr>
<td>C01</td>
<td>417</td>
</tr>
<tr>
<td>C18</td>
<td>378</td>
</tr>
<tr>
<td>C23</td>
<td>957</td>
</tr>
<tr>
<td>LB01</td>
<td>1150</td>
</tr>
</tbody>
</table>
Affinity-matrix surface capture

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

GS-CHO cell line, LB01
Manipulation of the transfection conditions results in a substantial increase in the number of transfectants.

Increasing the stringency of the selection conditions substantially increases the median antibody productivity.
Improving the fermentation process

- Significant potential to increase volumetric productivity of process
  - Maintain high viable cell concentration for extended period
    - Physicochemical environment (pH, temperature)
    - Medium design (including use of chemically defined media)
  - Feeding strategies
Physiochemical environment

- Control pH, temperature, dissolved oxygen concentration

- Small changes in pH can have a profound effect upon cell growth and productivity
  - Responses are cell line specific and can impact:
    - Maximum cell concentration
    - Time integral of viable cell concentration
    - Specific production rate
Effect of culture pH

Model GS-NS0 producing a recombinant antibody in a CDACF & PF bioreactor process

- Increased specific production rate: 0.59 pg/(cell·h) compared with 0.47 pg/(cell·h)
- Increased productivity: 590 mg/L compared with 240 mg/L
Medium design and feeding strategies

- Optimise basal medium
- Optimise feeds
- Maintain nutrient sufficiency
- Minimise waste product formation
Chemically-defined, animal component free and protein-free media (CDACF & PF)

- Increasing use of chemically defined media free of animal derived raw materials
  - Reduced risk of introducing adventitious agents
  - Improved process consistency and robustness (avoids potential variability of raw materials such as hydrolysates)
  - Benefits purification (reduced contaminant load)
Potential problems with CDACF & PF media

- Traditionally a lengthy procedure, often taking up to 16 weeks
- Often accompanied by transient poor growth and viability
- Potentially less productive than serum-free processes
Adaptation of a model GS-NS0 cell line to CDACF & PF medium

- Three process development iterations required
- First two failed either because too long or success rate too low
- Third iteration: 60 / 60 cell lines adapted within 4-7 weeks
Cryopreservation of CDACF & PF-adapted cells

- Removal of serum or BSA (and any other animal-derived component) from the cryopreservation mixture is highly desirable
  - Potential sources of adventitious agents
- CDACF & PF-adapted NS0 cell lines often showed poor viability and growth upon revival of cryopreserved cell stocks
  - Loss of process robustness
Cryopreservation of CDACF & PF-adapted cells

<table>
<thead>
<tr>
<th>CDACF &amp; PF medium</th>
<th>Serum in cryopreservation mixture</th>
<th>Culture viability prior to cryopreservation (%)</th>
<th>Culture viability upon recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Round 1</td>
<td>Yes</td>
<td>≥90</td>
<td>≤10</td>
</tr>
<tr>
<td>Round 3</td>
<td>Yes</td>
<td>≥90</td>
<td>≥90</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>≥90</td>
<td>≥90</td>
</tr>
</tbody>
</table>
Optimisation of a model GS-NS0 antibody process

Growth kinetics in a CDACF & PF bioreactor process

- Elapsed Time (h)
- Viable Cell Concentration ($10^5$ cells/mL)

- Original
- Iteration 1
- Iteration 2
- Iteration 3
- Iteration 4
Optimisation of a model GS-NS0 antibody process

Product kinetics in a CDACF & PF bioreactor process

- Cumulative Cell Time ($10^9$ cell h/L)
- Product Concentration (mg/L)

Graph showing product concentration over cumulative cell time for different iterations:
- Original
- Iteration 1
- Iteration 2
- Iteration 3
- Iteration 4
Process optimisation for a model GS-NS0

CDACF & PF 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>Q_p (pg/(cell·h))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum-free</td>
<td>640</td>
<td>476</td>
<td>0.74</td>
</tr>
<tr>
<td>Original protein-free</td>
<td>772</td>
<td>293</td>
<td>0.36</td>
</tr>
<tr>
<td>Iteration 1</td>
<td>1026</td>
<td>589</td>
<td>0.60</td>
</tr>
<tr>
<td>Iteration 2</td>
<td>1239</td>
<td>807</td>
<td>0.64</td>
</tr>
<tr>
<td>Iteration 3</td>
<td>1427</td>
<td>1035</td>
<td>0.71</td>
</tr>
<tr>
<td>Iteration 4</td>
<td>1405</td>
<td>1422</td>
<td>0.97</td>
</tr>
</tbody>
</table>
Downstream benefits of CDACF & PF medium for GS-NSO Cell Line

Purity of MAb at harvest

- Optimised protein containing culture: <30%
- Optimised CDACF & PF culture: 62%-76%
Optimisation of a GS-CHO Process

- Similar approach taken as with GS-NS0 cell lines
  - Fed-batch culture, initially using the same feed as the GS-NS0 process

- Suspension variant of CHO-K1 which grows in CDACF & PF medium without need for adaptation (can take several months)

- Improved selection of highly productive cell lines
GS-CHO growth characteristics

![Graph showing viable cell concentration (10^5/mL) over time (h) for GS-CHO growth characteristics.

- **22H11, iteration 1**
- **22H11, iteration 2**
- **LB01, iteration 2**
- **LB01, iteration 3**
- **LB01, iteration 4**

The graph indicates the growth characteristics of GS-CHO cells over time, with different iterations showing variations in viable cell concentration. The x-axis represents time in hours (0 to 400), and the y-axis represents viable cell concentration in 10^5/mL.
GS-CHO product accumulation

- 22H11, iteration 1
- 22H11, iteration 2
- LB01, iteration 2
- LB01, iteration 3
- LB01, iteration 4

Antibody (g/L) vs. Cumulative cell-time ($10^9$ cells.h/L)
## Process optimisation for a model GS-CHO

### CDACF & PF Bioreactor Process

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Process</th>
<th>Cumulative cell time (10^9 cell·h/L)</th>
<th>Antibody (mg/L)</th>
<th>Q_p pg/(cell·h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22H11</td>
<td>Original protein-free</td>
<td>267</td>
<td>139</td>
<td>0.52</td>
</tr>
<tr>
<td>22H11</td>
<td>Iteration 1</td>
<td>498</td>
<td>334</td>
<td>0.66</td>
</tr>
<tr>
<td>22H11</td>
<td>Iteration 2</td>
<td>1041</td>
<td>585</td>
<td>0.53</td>
</tr>
<tr>
<td>LB01</td>
<td>Iteration 2</td>
<td>2266</td>
<td>1917</td>
<td>0.89</td>
</tr>
<tr>
<td>LB01</td>
<td>Iteration 3</td>
<td>2493</td>
<td>2829</td>
<td>1.17</td>
</tr>
<tr>
<td>LB01</td>
<td>Iteration 4</td>
<td>2254</td>
<td>3560</td>
<td>1.55</td>
</tr>
</tbody>
</table>
GS-CHO process development timelines

Serum-free Process
Vector construction
Transfection
Adaptation and Selection
Lab-scale fermenter culture

Protein-free Process
Vector construction
Transfection
Adaptation and Selection
Lab-scale fermenter culture

Time (weeks)
Summary

- Number of approaches to achieving substantial process improvements
  - Cell line construction, process, potentially metabolic engineering

- Each approach gives increases, but synergistic improvements are possible

- Combination of efficient expression system (GS) and process optimisation gives high productivity for non-amplified NS0 and CHO cell lines

- Use of CDACF & PF media simplifies process optimisation and product purification

- Significant potential for further improvements based on process optimisation and cell line improvements