LakePharma’s CHO-GSN Platform for Stable Cell Line Generation

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Development Goal

- Objectives
  - CHO line with documentation and FTO to support commercial manufacturing
  - High yield $\rightarrow$ 3 gram per liter for antibody
  - Fast timeline
  - Consistency in performance

- Approach
  - GS selection on a CHO GS knockout line
History of CHO-GSN Host Cells

- Parental CHO K1 stock cells obtained by LP partner
- Knock-out of GS allele 1 by LP partner (CHO GS⁻/⁺)
- Knock-out of GS allele 2 by LP partner (CHO GS⁻/⁻)
- Suspension growth and adaptation
- Characterization of CHO GS⁻/⁻ and banking of CHO GS⁻/⁻
- Cells were analyzed using Infectious Microbe PCR Amplification Test (IMPACT) test panels I and III that aim to detect a wide range of human or other pathogens
- Results were negative for all infectious agents
- Via rAAV technology, both alleles of the catalytic domain of the Glutamine Synthetase (GS) enzyme were deleted
- Removal of the GS gene was confirmed both genetically and phenotypically
- Validation by reversion test when a functional gene copy of GS was restored by transfection
Stable Cell Line Generation Process

1 ~ 2 weeks

Target DNA

GS Stable Vector

Transfect CHO (MaxCyte)

GS Selection (MSX)

CHO Stable Pool

CHO Stable Clones

Subclones

RCB

5 weeks

10 weeks

Production Run (+3 weeks)

Production Run (+2 weeks)

Production Run (+2 weeks)
Case Study 1: CHO-GSN Pool and Clones of An Antibody

- HEK293 transient titer is 0.24 g/L
- CHO-GSN pool titer is 1.3 g/L
- Best clone titer is 4.5 g/L
Case Study 2: CHO-GSN vs. CHO-K1 of Fc-fusion

- GSN is the LP adapted suspension and serum free CHO GS^{-/-}
- CHO-K1 is the parental cell line (no knock-out, suspension)
- There is significant increase in titer when using the knockout line and even more increase when single cell cloning is performed

<table>
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<tr>
<th>Day</th>
<th>CHO-K1 pool titer</th>
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<tbody>
<tr>
<td>0</td>
<td>1.35</td>
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<tr>
<td>3</td>
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<td>4.5</td>
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<td>14</td>
<td>15.7</td>
</tr>
<tr>
<td>17</td>
<td>20.4</td>
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</table>
Case Study 3: CHO-GSN Producing A Bispecific Antibody

- A bispecific antibody does not contain Fc
- Titer quantification by Octet sensors
- Product related variants presented challenges in single cell cloning
- Through method development and custom screening approach, LakePharma was successful in generating a cell line expressing the bispecific antibody
Stable CHO-GSN Pools Have Higher Titer Than Transient
Case Study 4: Stability of Cell Line Producing an Antibody

<table>
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<tr>
<th>PDL</th>
<th>Antibody Yield (mg/L)</th>
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<tbody>
<tr>
<td>10</td>
<td>892</td>
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<tr>
<td>28</td>
<td>960</td>
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<td>60</td>
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</table>

CE-SDS

SE-HPLC

@ PDL 28

@ PDL 90
Case Study 5: Antibody Productivity Correlates with Gene Copy Numbers of Both Heavy and Light Chains

- Copy Number is Determined by qPCR Method
Highlights of LakePharma’s CHO-GSN Platform

- Complete cell line lineage and documentation; freedom to operate
- In-house proprietary cell lines and vectors
- MaxCyte electroporation
- High yield can be obtained at stable pool stage
  - Reduce the need to screen large number of clones for high yield
- Start to finish RCB in 6 months
- Proven success with difficult proteins
Additional Services Related to CHO-GSN Platform

- **Release Testing**
  - Sterility, mycoplasma, copy number, and viral testing

- **Stability Studies**
  - Stability over 80 generations

- **Scale-up Productions**
  - Large scale bioreactor capacity

- **Bioreactor Processing Development**
  - Process optimization, scale down and scale up