Accelerating Antibody Process Development: Exploring the Synergies Between Engineered Host Cells and Process Development

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Structure of Presentation

■ The GS System: The Background

■ Continuous Development of the System
  ■ Improved speed, efficiency and performance
    ■ Vector Development
    ■ Cell Line Generation
    ■ Production Process
      ■ Laboratory-Scale and Disposable Bioreactor Cultures

■ Summary
Disclaimer

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The actual results may differ materially in the future from the forward-looking statements included in this presentation due to various factors. Furthermore, Lonza Group Ltd has no obligation to update the statements contained in this presentation.

Note: All slides are incomplete without verbal comments.
The GS Gene Expression System™ Family

- GS System™ is a gene expression system used for commercial manufacture of therapeutic proteins using mammalian cells and at scales up to 20,000 L

- Underlying philosophy of GS System is that it is a commercial system
  - Select cell lines to fit a commercially-relevant platform

- GS System™ can be used with a number of parental cell lines
  - NS0
  - CHO
  - Sp2/0-Ag14
The GS System™: Adopted for Therapeutics at Every Stage

- Active GS licences for more than 370 products
  - ~140 products manufactured using GS System in active human trials
    - Seen by authorities in Europe, N America, Japan…..
  - 14 marketed products manufactured using GS System (6 GS-CHO / 8 GS-NS0)
- Used for products in both human and animal healthcare markets
GS System™ Family

- GS Gene Expression System™
  - Track record of 14 marketed products
  - Six products in GS-CHO, 8 in GS-NS0
  - Potelligent® CHOK1SV and NS0

- GS Xceed™
  - Developed from GS System™
  - Launched July 2012
  - Available for use globally including Asia
  - CHOK1SV GS-KO
Glutamine Synthetase (GS) Gene Expression System™ - Principles

- Many mammalian cells require exogenous glutamine
  - Native trait e.g. NS0
  - Engineered-in trait e.g. CHOK1SV GS-KO

- Complementation of glutamine auxotrophy used as basis of GS Gene Expression System™
  - Vector encoding product gene plus GS gene, allowing glutamine synthesis and selection of recombinant cells

- Glutamine synthetase is inhibited by methionine sulfoximine (MSX)
  - Used for selection for potential high producers
  - Allows use of GS System™ with GS positive cell types (e.g. wt CHO)
GS Expression Vectors

- Strong promoter (mCMV) drives expression of the gene(s) of interest (GOIs)
- Weak promoter (SV40) on GS gene is coupled with selection in high (stringent) levels of MSX
  - Selects for integration at transcriptionally active loci
- GOIs and GS gene are on the same plasmid and tightly linked
  - Selection for integration of GS gene into transcriptionally active loci results in co-integration of GOIs into same loci
  - Expression of linked product gene, driven by strong promoter, enhanced by favourable integration site
- Range of signal peptide candidates for secretion optimisation
GS-CHOK1SV & GS-CHO-KO Cell Lines in Laboratory-scale Bioreactors: Lonza’s Experience

- Antibody concentrations achieved in > 50 development projects
  - Product concentrations achievable defined by CDR.
  - No clustering by antibody isotype (IgG1, IgG2 or IgG4).
  - Choice of Fc should be for desired effector function rather than for any productivity concern.
Stability of Productivity for Cell Lines Derived from GS Xceed™ System

- Antibody producing cell lines created using CHOK1SV GS-KO host were evaluated at an early stage
- Maintained MSX-free medium
- Productivity monitored every 15 to 20 generations in fed-batch shake-flask model
- 7 / 9 cell lines would be considered suitable for manufacturing
  - Change in mAb concentration across manufacturing window was in range [-20%, 20%]
- To-date 18 / 24 (75%) cell lines would be considered stable
  - 2 mAbs
Consistent Product Characteristics in Cell Line Stability Studies

- **CHOK1SV**
  - Data from 43 mAbs
  - No changes in PC observed

- **CHOK1SV GS-KO**
  - Data from 1 mAb, 6 cell lines
  - No change in PC observed across study
    - Size variants (reduced and non-reduced SDS-PAGE)
    - Charge variants (IEF)
    - Monomer and aggregate proportions (GP-HPLC)
    - Glycan profiles (MALDI-TOF-MS)
GS-CHO Platform Process

- Output of long-term, on-going continuous improvement project
- Fed-batch process designed specifically for GS-CHO cell lines
- Comprises: process parameter recommendations, media, and feeds
- Animal component-free, no hydrolysates, no proteins or polypeptides
  - CHOK1SV and its derivatives do not require insulin or Long-R³
- Integral part of the GS Xceed™ System
cGMP Manufacturing: Influence of GS-CHO Process on Harvest Concentration

- Stepwise improvements seen as Lonza platform evolves from Version 6 to Version 8.

- Data for each cell line are mean values from \( n \geq 1 \) runs and 60 different mAbs

- cGMP manufacturing bioreactors (200 L to 20,000 L)

- Harvested no later than 15 days after inoculation (day 15 is routine day of harvest for GMP manufacturing)
GS Xceed™ System: Performance of rec-GS-KO Cell Lines at Manufacturing Scales

- GS Xceed™ cell line evaluated at three scales
- Comparable product accumulation profiles and concentrations at harvest
Continuous Improvement in Vector Design, Cell Line Selection and Process Development
Vector Development

- GS Xceed™ System vectors are created as a single final vector for transfection
  - Two-step cloning process for mAbs or other two chain proteins.
  - Approaches can be employed to create GS expression vectors in a single step to reduce timelines.
Cell Line Development: Selection Strategies

**SELECT EARLY**
- Requires prediction of manufacturing behavior at very early stage
- Good predictive markers

**SELECT LATE**
- Selection occurs in manufacturing process and scale
- Requires assessment of large numbers of cell lines in manufacturing plant
- Lengthy and resource intensive
- Impractical

**COMPROMISE**
- Multiple steps using scale-down bioreactor models
- Economical
- Compatible with resources
GS Xceed™ System – Fast CLC&D

- 17 weeks from transfection to lead cell line, incl. selection for fit to commercial platform process.
- 200 to 1,000 clones; product concn; MWP
- 200 to 500; fit to media & feeds, product concn; DWPs
- 9 to 20; fit to inoculum process; E-flasks
- 9; growth; product concn & characteristics, etc; Ambr™
Selection Strategies: Finding the “Special One’

- Selection strategies identify high ranking cell lines
- Only census of all cell lines will find top ranked \( n \) cell lines
- Rank position of selected cell lines varies considerably between rounds
- Highest ranked cell lines can be ranked low in earlier rounds
- Progress as many as possible between rounds

Identifying High Performers with Reduced Screening

- For any screening round, probability density function describes distribution of product conc

- Biologically, upper limit to possible values

- No. of cell lines = \( \rho(\text{good cell line}) \times \text{no. screened} \)
Some of Lonza’s Antibody cGMP Experience With GS-CHO Cell Lines

- 56 different mAbs produced in cGMP facilities to-date
  - Excludes mAbs that did not progress beyond pilot-scale
  - Mix of IgG1, 2 & 4
  - Number of process iterations
  - Ph I to Ph III
- 75% of cell lines ≥2 g/L mAb
- 6% of cell lines ≥5 g/L mAb
Distribution of Harvest mAb Concentrations at Laboratory-Scale: Large & Wide Data-Set

- Using very simple model based upon cGMP and lab-scale data
  - Under-estimate screen size
- Cell line making at least 5 g/L, need to screen at least 135 to find one, on average
- ≥ 2 g/L, need to screen 3!

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P(\text{mAb} \geq 5 \text{ g/L}) = 0.12
\]

\[
P(\text{mAb} \geq 2 \text{ g/L}) = 0.54
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Selection Strategies with GS Xceed™ System

- High probability that generate cell lines capable of achieving minimum mAb concentration needed to supply clinical trial
- GS System™ capable of generating and isolating very high mAb producing cell lines
  - 6+ g/L, 80 – 95 pg/(cell·day)
- Consequently, large numbers of cell lines in range 2 – 4 g/L can be found
  - Caveat: mAb is inherently capable of achieving these concentrations
- Results:
  - Further development of cell line not needed
  - Process re-engineering shortens timeline above-and-beyond reductions achieved by biology
Improved Work-Flow for Selection of Clonal Cell Lines

1. Automated or manual colony sampling
   - Productivity assessment: 616 wells
   - Shaking 96-DWP

2. Fed-batch productivity assessment: 24/48 cell lines
   - Week ~11: choose 8 candidate cell lines
   - Week ~12: Cryo-preserve RCB
   - Week ~14: Choose lead cell lines Start cGMP MCB

Two rounds of screening

- Transfect CHOK1SV GS-KO host cells with vector
- Generate pools of transfectants
- Clone by FACS
- Automated colony identification
- 160 plates

GMP cell banking

Optional PQ & productivity screen: 8 cell lines

Transfect CHOK1SV GS-KO host cells with vector

Automated colony identification

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GMP cell banking

Optional PQ & productivity screen: 8 cell lines
Improved Bioreactor Processes

- Process understanding
  - Greater process understanding required by QbD applies to development of platform processes
- Stepwise iterative approach used to develop of the Version 8 process.
  - Further development of bioreactor processes currently using sophisticated DoE approaches
    - Increase productivity, increase robustness & resilience, reduce complexity
- Enhancing performance of a disposable bioreactor process
Product Profiles for ‘Model’ GS-CHO Cell Line LB01 in Laboratory-scale Bioreactors. Comparison between the v6, v7 & v8 processes

Viability at day 15
- v6: 90%
- v7: 79%
- v8: 43%

Process extended beyond the current routine harvest day for GMP manufacturing culture (day 15)
Improved Disposable Bioreactor Processes

- Disposable laboratory scale bioreactor cultures used for material generation for R&D and Development activities using the GS Xceed™ system.
- Improvements to physiochemical culture environment resulted in improved culture performance.
  - Achieved >2-fold increase in product concentration at harvest.
Next Generation GS Xceed™ Process

- Current development programme
  - Multiple stages and experimental rounds within stages
- Exemplar of experimental design
  - 11 factors, each at multiple levels, studied in 96 reactors
  - 54 responses (cell, product & metabolite concentrations)
- Development achieved in a reduced timeline due to use of automation.
- Experiment execution not possible in a commercially reasonable time-frame using reasonable resources without use of automation.
Process Development for GS Xceed™ Platform

- Use of automation brought process understanding efficiently
- Understand how the 11 factors define the process design space
- And output was predictive of behaviour at laboratory-scale
Summary

- GS Xceed™ System available for use in all major pharma-biotech markets **globally**
- Select cell lines for commercial manufacturing at 9 to 17 weeks after transfection
- Improved stability
- Cell lines are screened to fit GMP production process
- Philosophy of making continuous improvements to the system
- Vectors
- Cell line development strategies
- Production processes