Cell Line Development: 
Taking Biological Therapeutics from DNA to Production
6th Annual Cell Line Development & Engineering Asia

Teh Song Hooi | May 2017
Disclaimer

Certain matters discussed in this presentation may constitute forward-looking statements. These statements are based on current expectations and estimates of Lonza Group Ltd, although Lonza Group Ltd can give no assurance that these expectations and estimates will be achieved.

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
Mammalian Production and Development Sites on Three Continents

- **Portsmouth, NH (USA)**
  - Mammalian Cell Culture
  - 5,000L to 20,000L cGMP

- **Porriño (Spain)**
  - Mammalian Cell Culture
  - 4 x 10,000L cGMP

- **Slough (UK)**
  - Mammalian Cell Culture
  - 200L to 2,000L cGMP
  - Process R&D Services

- **Tuas – Singapore**
  - Mammalian Cell Culture
  - 200L to 20,000L cGMP
  - Process R&D Services
  - Cell Therapy

View our 360° virtual tours at [www.lonzavirtualtours.com](http://www.lonzavirtualtours.com)
Lonza Singapore

- Full range of Mammalian Cell Culture Operations
- From DNA to commercial cGMP product
  - Cell Line Creation
  - Process Development
  - Analytical Services
  - Technology Transfer
  - Manufacturing for Clinical Development
  - Manufacturing for Commercial Operations
- Cell Therapy Operations
Cell Line Development: Taking Biological Therapeutics from DNA to Production

- Selection Strategies

- What are the considerations to select a ‘desirable’ cell line with ‘right’ product?

- Future Perspectives:
  - Cell Line: Site-Specific Integration
  - Protein Sequence Variant Analysis
Cell Line Development

Selection Strategies

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

**TIME**

**Large-scale Bioreactors**
- **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
Cell Line Development

Considerations for a ‘desirable’ cell line producing ‘right’ product

1. Expression Vectors
   - Choice of primary amino acid sequence, selection marker (GS, dhfr, antibiotic), vector architecture (promoters, enhancers), gene sequences (gene and signal peptide optimization) and beyond

2. Host cells
   - CHO, NS0, human (HT1080, HEK293 and Per.C6®) etc.

3. Process for selection of recombinant cell lines for production
   - Define a suitable approach to screen high numbers of cell lines for favorable characteristics
   - Transfection conditions, cloning strategy and production processes at different scales
Strong promoter to drive expression of GOIs

- Virus; elongation factor

Increased copy number of GOIs that give proportional increase in gene expression

- Co-amplification of GOIs and selectable marker gene (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

Insertion of expression vectors into transcriptional active loci

- Site-specific integration
- Selection system
Expression Vector

The GS Gene Expression System™

- 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™ is used at Every Therapeutic Stage

- Active GS licenses for more than 500 products in clinic
- ~230 products manufactured using GS system in active human trials
- 33 marketed products manufactured using GS system (20 GS-CHO / 13 NS0)
Expression Vector
The GS Gene Expression System™

- 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
Variants of CHO cells are most commonly used

Proprietary CHO cell lines developed with characteristics suited to manufacturing therapeutic proteins

- Potentially more potent product
  - $\alpha$-1,6-fucoysltransferase ($FUT8$) gene knockout (KHK Biowa, KHK Biowa & Lonza)
  - 1,4-N-acetylglucoaminyltransferase III ($GnTIll$) knock-in (Roche Glycart)
  - GDP-6-deoxy-D-lyxo-4-hexulose reductase (ProBiogen)

Pre-adapted to manufacturing environment

- Grows as single cell suspension in CDACF media (Wyeth, Lonza, SAFC, etc)
- Uses CDACF media at all stages of cell line construction process, not just manufacturing scales

Exhibits good growth characteristics

- High average viable cell concentration, low doubling time

Compatible with high specific production rates

- Mitochondrial potential, plenty of ER (Hu et al. 2013, Biotechnol Prog, 29:980)
Host Cells

CHOK1SV

- CHOK1SV developed as suspension variant of CHO-K1 (2003)
  - Grows as single cell suspension
  - Pre-adapted to growth in CDACF media
  - Exhibits good growth characteristics
  - Reach high maximum viable cell concentration
  - Able to maintain cultures at high culture viability
  - Many licensed products manufactured using CHOK1SV

- Selection of CHOK1SV cells transfected with GS expression vectors
  - Use of protein-free CDACF media is possible at all stages of cell line construction process
  - Sorting using FACS etc. is possible
  - Fast suspension re-adaptation after transfection/cloning

- CHOK1SV scaled-up to 20,000 L
Host Cells
CHOK1SV GS-KO

- Genetic engineering to disrupt the endogenous GS gene in the CHOK1SV cell line
  - Selection of resultant clone with appropriate manufacturing characteristics
  - Preparation of fully tested and characterized GMP cell banks

- No selective agent required in routine culture
  - Faster growth = reduced timeline, improved cell line stability characteristics

Polyclonal anti-rat GS (Sigma); 20 µg protein / lane - comparable loading per lane by anti-actin staining and ECL detection

<table>
<thead>
<tr>
<th>CHOK1SV</th>
<th>XF3 (GS +/−)</th>
<th>XF3-29B7 (GS+/-)</th>
<th>GS-/- CHOK1SV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>53A4</td>
<td>54C3</td>
<td>54F8</td>
</tr>
<tr>
<td></td>
<td>55F12</td>
<td>55B7</td>
<td></td>
</tr>
</tbody>
</table>

Transfection & pool selection
- MSX removed
- Cloning
- Suspension evaluation
- Identify candidate cell lines
- Suspension expansion
- Development cell stocks
- v8 manufacturability
- Identify final lead cell line

PharmaBiotech | Teh Song Hooi | May 2017
Host Cells

CHOK1SV GS-KO

Stability

GS Xceed™ (red diamonds)
- Data from 9 mAb & 3 recombinant proteins (97 cell lines) maintained in the absence of MSX
- 68% clones stable
  - Change in [mAb] across manufacturing window (± 20%)

CHOK1SV (blue diamonds)
- Data from 44 mAbs (140 cell lines) maintained in the presence of MSX
- 62% clones stable
Host Cells

**CHOK1SV GS-KO**

Consistent Product Characteristics in Cell Line Stability Studies:

**CHOK1SV and CHOK1SV GS-KO**

- Data from 44 mAbs
- No changes in PC observed
- Example study design
  - Size variants (reduced and non-reduced SDS-PAGE)
  - Charge variants (IEF)
  - Monomer and aggregate proportions (GP-HPLC)
  - Glycan profiles (MALDI-TOF-MS)
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

Improved Models of Production

Application of fed-batch processes early in CLC enables better decisions to be made

- <2 mL 96-DWP culture and productivity assessment
- Practical way to test >200 cell lines
Improved Models of Production

Miniaturized bioreactors

- Enable process control at $\leq 15\, \text{mL}$ scale
- Better production format than a shake-flask
- 48 station system in place in Lonza Singapore
Lonza GS-CHO Platform Process

Principles Applied

- Productivity enhancement
  - Minimise unproductive time in the reactor
  - Maximise Qp of cell lines selected and reduce max VCC

- QbD: design space characterisation to improve process robustness
  - Risk Identification and Analysis
    - Cause and Effect Analysis
  - DOE approach using response surface IV-optimal design methods
    - Investigate a larger proportion of the design space
  - Multivariate data analysis
    - PCA

Improved Process

- Derived models used to optimise simultaneously key targets including
  - Maximise viability at harvest
  - Maintain antibody concentration at harvest
  - Minimise sensitivity of process to variations in process inputs

- The optimised process was tested in 10 L airlift bioreactors

Racher (2014) 10th Cell Line Development & Engineering Meeting, Berkeley, Ca
Objectives

- Process simplification
  - Keep benefits of more-complex process but hide complexity from end-users
  - Reduce opportunities for process errors and deviations
- Minimise batch-to-batch variability
- Improve scaleability

How?

- Use of propagation-of-error techniques
- Methods find high plateaus or broad valleys where performance peak is unaffected by deviations from factor level set-points
Lonza GS-CHO Platform Process

Experiment Outline

- Critical factor identification
  - One-factor-at-a-time > Design space identification > 10 L

- Scalability
  - Ambr15, 10 L STR, 400 L STR, 1 kL SUB

- Robustness
  - Naïve users at second site (5 L STR)
Lonza GS-CHO Platform Process

Outcome

- Process variability from noise around parameter set-points reduced
- Process simplified
  - Naïve users successfully ran process
- Comparable performance across scales and bioreactor types
- Improvement in productivity
Cell Line Development

Work-flow for Selection of Clonal Cell Lines

1. **Generate pools of transfectants**
   - CHOK1SV GS-KO

2. **Clone by FACS**

3. **Automated colony identification**

4. **Colony sampling**

5. **Colony selection**

6. **Upstream process dev.**

7. **Bioreactor studies/scale up**

8. **Stability for manufacturing**

9. **GMP cell banking**

10. **RCB**
   - 8 cell lines

11. **Fed-batch productivity assessment**
   - 48 cell lines

12. ** optional abridged fed-batch productivity assessment (96-DWP) **

13. **Expand to shake-flasks <120 cell lines**

14. **Productivity assessment**
   - 200 to 1000 cell lines
Good number of ‘desirable’ cell lines producing ‘right’ product

Cell line construction programme generating cell lines with manufacturing characteristics:

- Acceptable growth
- Productivity
- Stability

Scalable process

Outcome:

- Flexibility in CLC and Spring Forward!

Note to reviewers:
New slide – containing customer project data showing ambr15 to 400L SUB – scalability of Lonza’s process and flexibility in CLC.
Scope of Talk

Cell Line Development: Taking Biological Therapeutics from DNA to Production

- Selection Strategies
- What are the considerations to select a ‘desirable’ cell line with ‘right’ product?

Future Perspectives:
- Cell Line: Site-Specific Integration
- Protein Sequence Variant Analysis
Site-Specific Integration (SSI)

Premise
- Pre-identification of a chromosomal site supporting high-level, stable expression
- SSI technology is used to accurately deliver an expression vector into this site

Potential advantages
- Reduced cell line screening effort – 10 rather then 100s or more
- Consistent, reproducible transgene expression
- Predictable cell line performance: Growth, Productivity, Stability

Process advantages
- Shorter development timelines
- Lower resource utilization
- Platform process – Media, feeds etc.

A potential disadvantage
- Lower expression levels
  - Single or low vector copy number
  - Low Qp cell lines
Incorporation of FRT Sites at Transcriptionally Active Loci Within the CHOK1SV Genome

Phase I (Developing a Founder Cell Line)

- Incorporation of FRT sites at transcribed loci
- Random cell line process

Gene Copy Analysis

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>HC average copies/cell</th>
<th>LC average copies/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>1G11</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>11A7</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

Clone 11A7 was chosen for the development of SSI host

- Two heterospecific FRT sites, wild-type (F) and mutant FRT (F5)
Development of an SSI Host Cell

Introducing a ‘Null’ Expression Cassette Into the FRT-tagged Loci by RMCE – Phase 2

Integrated mAb Probe Vector

Flp + Pur⁺

(Recombinases Mediated Cassette Exchange)

Predicted configuration of the FRT-tagged loci (“Landing Pad”) in the SSI host cell line
Characterization of the ‘Landing Pad’ in 10E9

- To confirm the Landing pad structure the genomic locus containing the vector was characterized by
  - Southern blot
  - Northern blot
  - mRNA PCR analysis
  - Next generation sequencing

- ‘Landing Pad’ contains two copies of recombination sites to allow for insertion of one (1X) or two (2X) expression Cassettes
Generation of mAb-Expressing Clones by Consecutive RMCE
SSI Pools Achieved Consistently Across Six Projects

- Three independent transfections performed for each project
  - Pools independently evaluated in fed-batch shake flask cultures
  - Note: For comparison – parental mAb-expressing GS-CHO cell line 11A7 produced ~3 g/L in Fed batch shake flasks
SSI for Potential Rapid Material Supply

8 weeks from transfection to purification

3 x Tfx

Selection
~3 weeks

Expansion
~2 week

Disposable 50 L
~1 week

250L SUB
2 weeks

After Mott J. (Pfizer) 5th Bio Innovation Leaders Summit, London 2012
Mitigating Product-related Risks Protein Sequence Variants

- Possible sources, single or interactions
  - Cell line (tRNA profiles; mutation frequency)
  - Manufacturing process (availability of amino acids)
  - Transgene (presence of mutations; codon frequency)

- Potential consequence is increased immunogenicity
  - Depends upon amino acid change
  - Most commonly described risk

- How is occurrence of protein sequence variants dealt with?
  - Standardised cut-off methods for variant abundance assume cell lines or processes producing higher frequencies (typically >0.5%) are not acceptable
  - Ignores risk that some mutations are potentially more immunogenic
MS-based Product-sequence Variant Analysis
Cell Line Stability Study (1)

- Variant detected in one cell line in the panel
  - Comparative analysis of MS data

- Variant present only at high-generation numbers: present in both lineages

- Targeted analysis allowed unambiguous assignment

- Quantitation
Quantification showed variant at ~1% abundance
- At this level, variant was eliminated and not risk assessed
- Other examples show that single amino acid changes increased immunogenicity

Nucleic acid sequence analysis showed mutation present at higher generation number but not 70 generations earlier

<table>
<thead>
<tr>
<th>Generation number</th>
<th>Relative abundance (mean ± SD)</th>
<th>Lineage A</th>
<th>Lineage B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>Late</td>
<td>0.98% ± 0.02%</td>
<td>1.69% ± 0.02%</td>
<td></td>
</tr>
</tbody>
</table>
Protein Sequence Variant Analysis for Mitigating Product-related Risks

- Sequence variant analysis earlier in development reduces probability that cell line making a “less safe” protein is selected.

- Data from 18 cell lines in 3 in-house GS-CHO construction projects:
  - No sequence variants have to date been detected at early generation number.
  - One sequence variant, due to mutation in transgene, detected at 1% abundance in higher generation number cells.
  - Estimated incidence rate is 6%.
Summary

- A reliable system in molecular, cell culture and production process to take biological therapeutics from DNA to production:
  - Expression System: GS Gene Expression™ System
  - Host Cell Lines: CHOK1SV GS-KO
  - Production Processes: GS-CHO Platform Process

- Big reductions in time from DNA to Production have already been made
  - Shorter timelines mean fewer opportunities to solve problems

- Greater use of methods that identify and mitigate risks improves our ability in cell line development to generate a ‘desirable’ cell line producing the ‘right’ product
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Thank You

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