

Cell Line Development and
Engineering Meeting

Lonza

Suitability and Stability of Cell Lines for Manufacturing Antibodies: Experience with GS Cell Lines

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Scope of Talk

- Background
- Assessment of Stability and Suitability, Lonza's Approach
- Experience with Antibody Production using GS Gene Expression System
 - GS-NS0 Cell Lines
 - GS-CHO Cell Lines
- Improved Process Fit
- Summary

Background

Regulatory Guidance - ICH Topic Q5D

- Demonstrate suitability of manufacturing process
 - Evaluate at a minimum of two timepoints
 - Minimal number of population doublings
 - At or beyond the limit of *in vitro* cell age for production

- Cell substrate stability
 - ‘Consistent production of the intended product of interest’ by a characterised cell bank (WCB)
 - Focus on consistency of production
 - Use protein biochemistry techniques to characterise product quality

- Growth characteristics and productivity characteristics ‘may be useful’ for assessment of cell substrate stability

Cell Substrate Stability is....

- From a regulatory viewpoint, key criterion for assessment of stability
 - Production of product with consistent characteristics
 - *Not consistent growth and productivity characteristics*
 - *Consistent growth and productivity are important for process economics and process robustness*

- Stable recombinant cell line
 - Consistent product characteristics between cells at a minimal number of population doublings and at or beyond limit of *in vitro* cell age for production

Cell Substrate Stability is not....

- From regulatory viewpoint, cell substrate stability is not consistent growth and productivity characteristics
- Contrasts with scientific literature
 - Cell line stability linked to understanding how copy number, mRNA levels, and vector integration site influence productivity changes with increasing cell age

Assessment of Stability and Suitability, Lonza's Approach

Assessing the Stability of GS Cell Lines (1)

- Grow cells in a good scale-down model of the inoculum and production cell culture processes for required number of population doublings
 - Each cell line serially sub-cultured using inoculum process
 - At suitable interval, assess cells in model of production process

Assessing the Stability of GS Cell Lines (2)

- Analyse Protein A-purified antibody from different points in the study
 - Current methods may include peptide mapping, MALDI-TOF-MS, ESI-MS, SDS-PAGE and IEF electrophoresis, activity assay
 - Antibody quantified by ELISA or quantitative Protein A HPLC

- Trend changes in cell line characteristics
 - Viable cell concentration and IVC
 - Antibody concentration and specific production rate (Q_p)

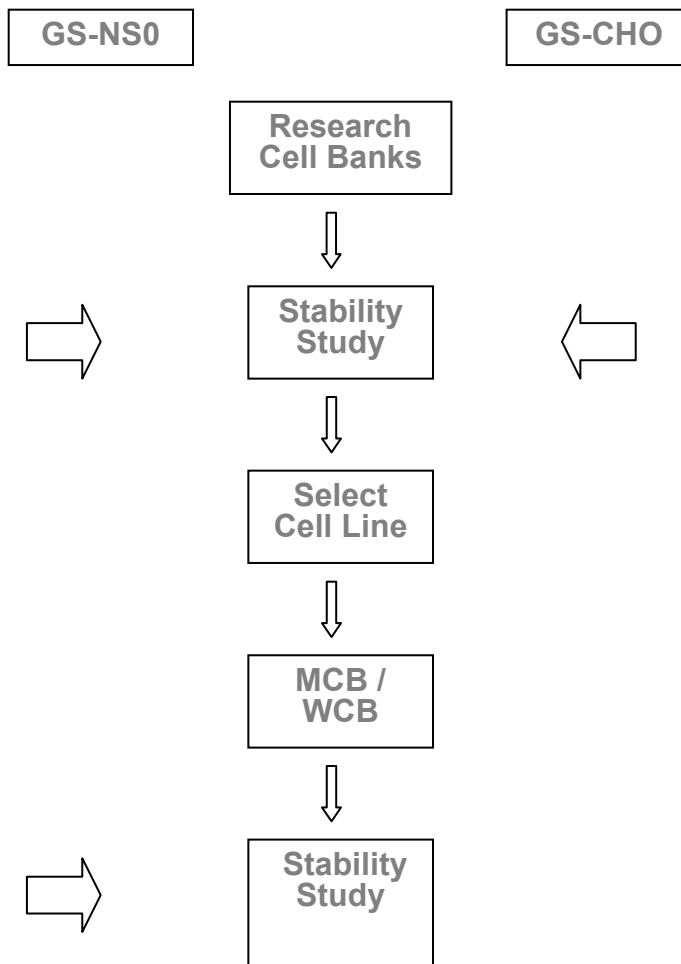
Stable and Suitable Cell Lines

- Stable cell line
 - Product must be biochemically comparable, *using the analytical techniques available*, at the beginning and end of the study

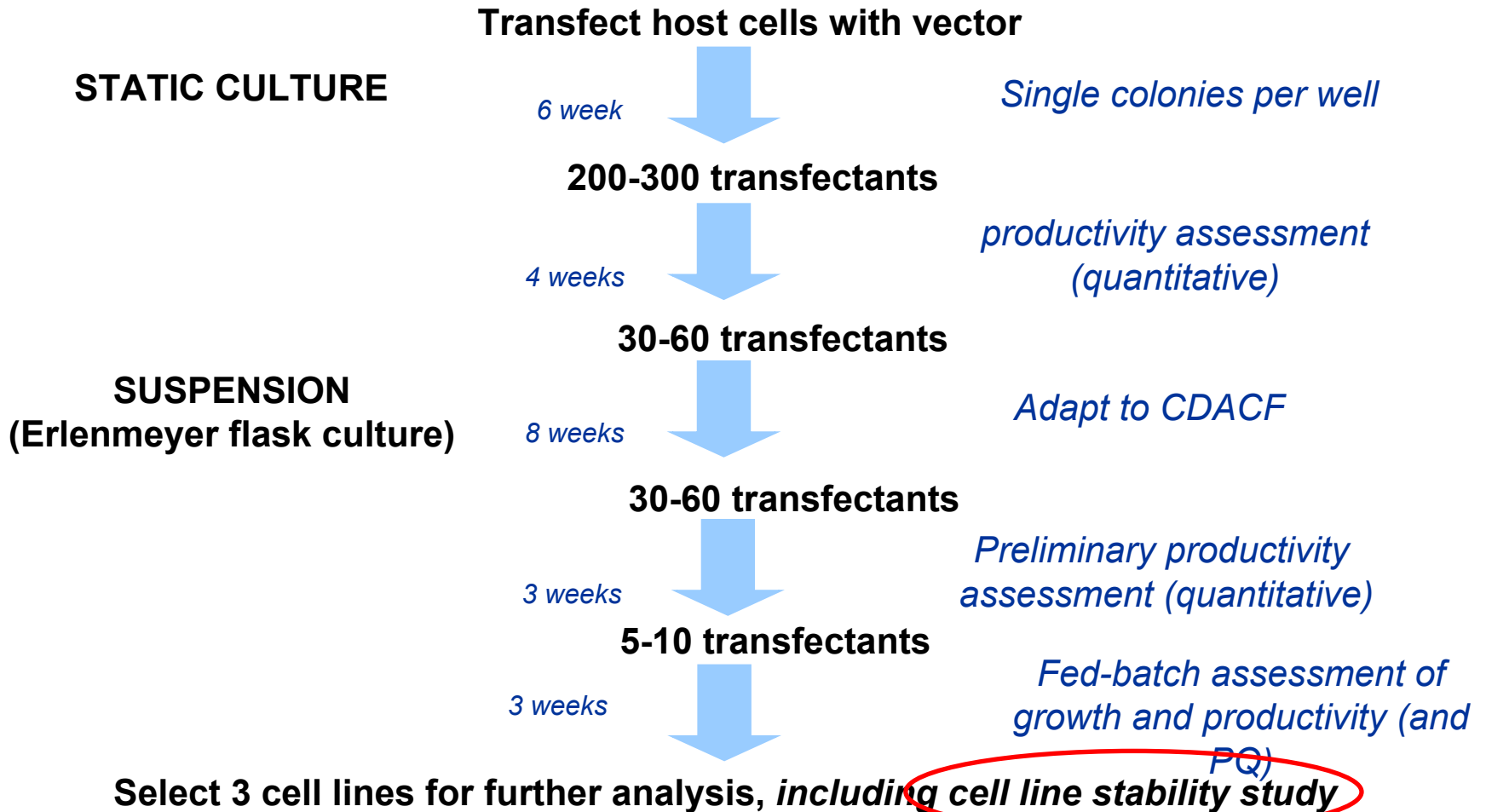
- Cell line suitable for use in manufacturing process
 - Change in antibody concentration across the 40 population doubling window between MCB / WCB and limit of *in vitro* cell age less than 30% (*internal Lonza guidance*)
 - Twice expected CV of ELISAs used in some early programmes

Experience with GS Cell Lines

Data Sources



Selecting High Producing Cell Lines: Strategy selects against cell lines exhibiting gross changes in productivity



Studies with GS-NS0 Cell Lines

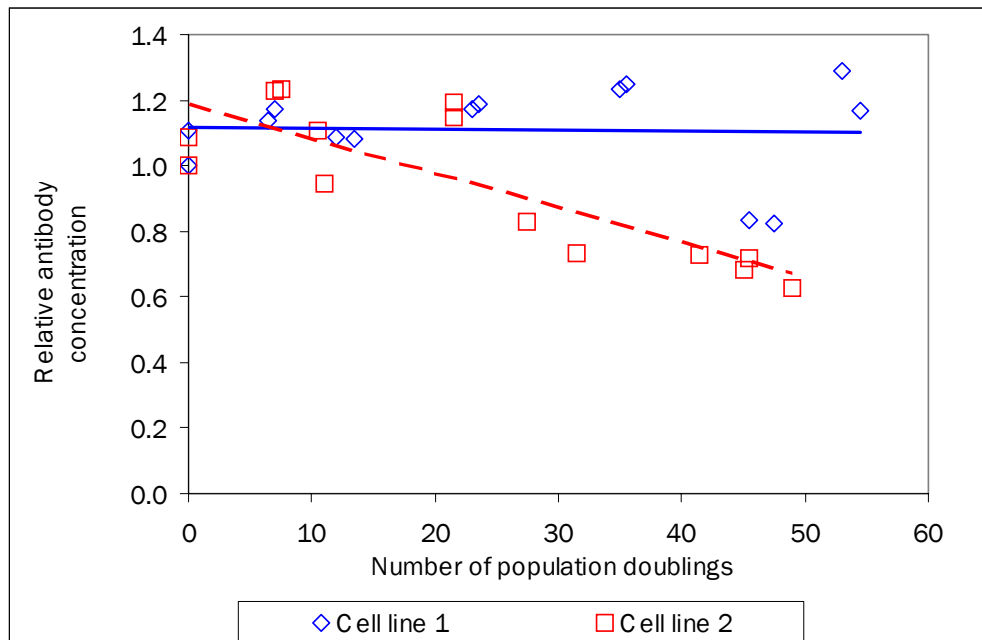
- Data from randomly selected GS-NS0 cell line development programmes undertaken over the last 10 years
 - 23 antibodies
 - 61 different cell lines
- No gene amplification
- Selection pressure: use of glutamine-free media, no MSX
- Cell lines adapted to either serum-free or CDACF media

Analysis – GS-NS0

- Stable cell line
 - Product is biochemically comparable, using available analytical techniques, at start and end of study
 - To ensure consistency over review period, only electrophoretic data considered
 - Banding pattern at start and end of study are comparable

- Suitable cell line
 - Change in antibody concentration across 40 pd manufacturing window (between WCB and limit of *in vitro* cell age) not greater than 30% (*internal Lonza guidance*)

Suitability of GS-NS0 Cell Lines



- Is there a substantial change in the parameter value across the manufacturing window?
 - Cell line 1: 1%
 - Cell line 2: 36%
- Cell line 1 is suitable
- Cell line 2 not considered suitable as change too big

Changes in Biochemical Comparability with Cell Age for GS-NS0 Cell Lines

Number of cell lines screened	Number of cell lines where major changes in the banding pattern on SDS-PAGE and IEF gels were observed	Proportion
47	3	0.06 (3 / 47)

- Data available from 47/61 cell lines
- Changes seen for 3/47 (0.06) cell lines
 - In each instance, there was an alternative cell line available that was used instead
- Cell lines exhibiting changes in product quality with increasing population doubling number are infrequent

Change in Antibody Concentration with Cell Age for GS-NS0 Cell Lines

Condition	Proportion
No change in antibody concentration	0.79 (48 / 61)

- Large proportion of cell lines do not show change in antibody concentration with cell age

Changes in Antibody Concentration and Qp with Cell Age for GS-NS0 Cell Lines

		Antibody concentration		
		[30% change	>30% change	
Qp	[30% change	0.69 (40 / 58)	0.05 (3 / 58)	0.74
	>30% change	0.09 (5 / 58)	0.17 (10 / 58)	0.26
		0.78	0.22	1.00

- Antibody concentration and Qp consistent for most cell lines
- Change in Qp main cause of change in antibody concentration
- Changes in cell growth may be neutral or deleterious

Studies with GS-CHO Cell Lines

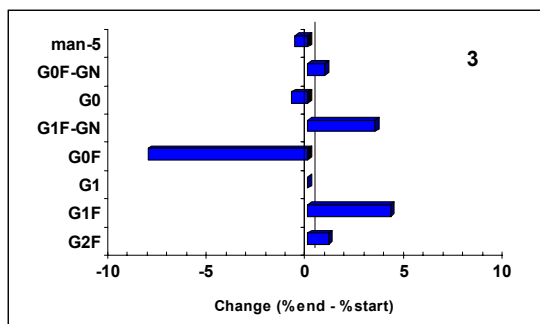
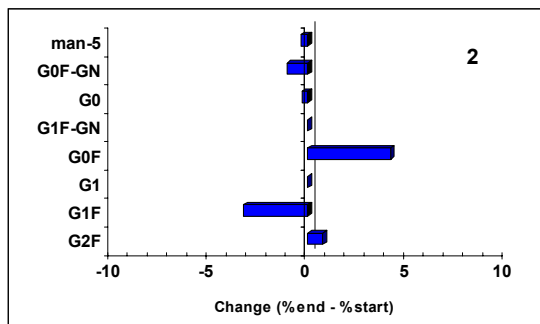
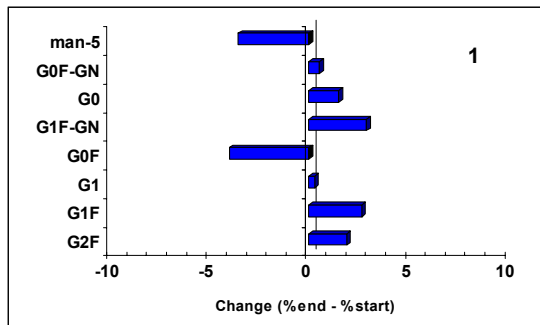
- Data from GS-CHO cell line development programmes undertaken over the 2 years
 - 5 antibodies
 - 15 different cell lines
 - CHOK1SV host
- No gene amplification
- Selection pressure: use of glutamine-free media with MSX
- Cell lines adapted to CDACF medium

Analysis – GS-CHO

- Stable cell line
 - Product is biochemically comparable, using available analytical techniques, at start and end of study
 - SDS-PAGE and IEF banding pattern are comparable
 - Oligosaccharide profiles (MALDI-TOF-MS) are comparable

- Suitable cell line
 - Change in antibody concentration across 40 pd manufacturing window (between MCB / WCB and limit of *in vitro* cell age) not greater than 30% (*internal Lonza guidance*)

Oligosaccharide Profiling – Examples from GS-CHO Cell Lines



- Figures show differences in proportions at start and end of study
- Cell lines 1 and 2
 - Differences are within expected assay variance
- Cell line 3
 - Most differences are within expected assay variance
 - Difference G0F is slightly larger than expected variance: the significance of this is currently uncertain
- Overall, glycosylation of antibody considered comparable between start and end

Changes in Biochemical Comparability with Cell Age for GS-CHO Cell Lines

Number of cell lines screened	Number of cell lines where major changes in the banding pattern on SDS-PAGE and IEF gels were observed	Number of cell lines where major changes in oligosaccharide profiles seen
15	0	0

- Data available from 15 cell lines
- Changes seen for none of cell lines tested
- Cell lines exhibiting changes in product quality with increasing population doubling number are infrequent (frequency $<1/15$)

Changes in Antibody Concentration and Qp with Cell Age for GS-CHO Cell Lines

		Antibody concentration		
		[30% change	>30% change	
Qp	[30% change	0.867 (13 / 15)	0.000 (0 / 15)	0.867
	>30% change	0.067 (1 / 15)	0.067 (1 / 15)	0.133
		0.933	0.067	1.000

- Antibody concentration and Qp consistent for most cell lines

Improved Process Fit

Cell Line Suitability

- Loss of Productivity
 - Lower selective pressure
 - By-pass selection
 - Inherently unstable cell line
 - Influence of culture conditions

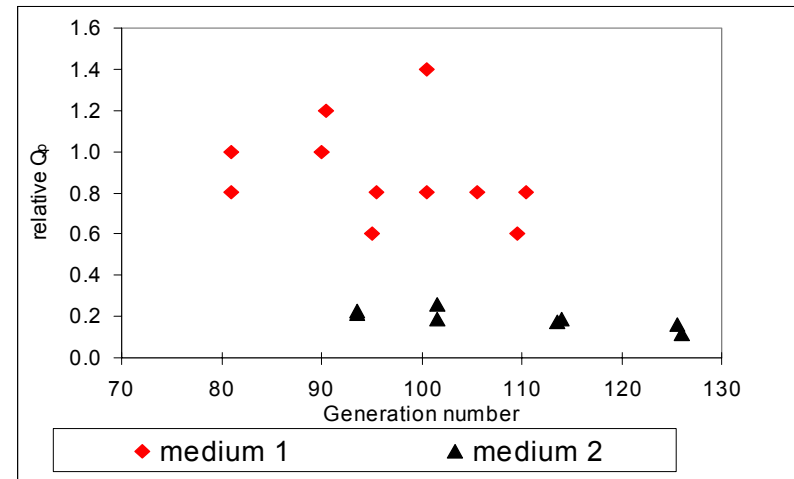
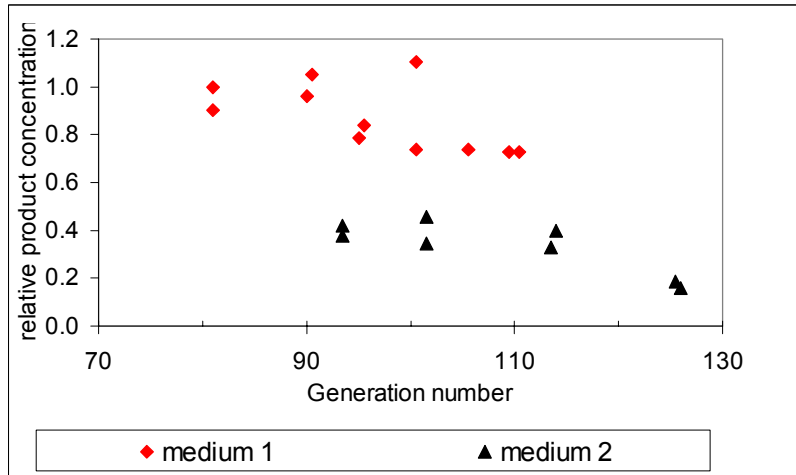
- How can loss of productivity be slowed or prevented?
 - Two examples with GS cell lines

Impact of Subculture Day upon Behaviour of a GS-NS0 Cell Line

Subculture day	Maximum viable cell concentration	Integral of viable cell concentration	Antibody concentration	Specific production rate
4	1.00	1.00	1.00	1.00
5	1.22	1.12	0.99	0.90
6	1.60	1.12	1.20	1.05

- GS-NS0 passaged from single stock culture, for about 35 population doublings, with different subculture regimes and then evaluated in batch culture
- Data normalised to value from day 4 regime batch culture
- Volumetric productivity maintained by splitting culture later
 - Combination of better growth and higher specific production rate

Influence of Medium Composition upon Behaviour of GS-CHO Cell Lines



- Cells passaged in 'weak medium' (medium 1) or 'rich medium' containing high levels of GS supplements (medium 2)
- Medium supplements probably allowed cells to by-pass MSX selection, resulting in decline in GS activity
 - As GS and transgenes linked, get concomitant decline in Q_p

Summary

Stability and Suitability of GS Cell Lines

- Cell lines exhibiting changes in product quality occur infrequently
- About 80% of GS cell lines did not exhibit productivity changes
 - Typically select 3 cell lines per programme
 - Probability that all three cell lines unsuitable $p \approx 0.01$
 - On average, expect to observe 3 unsuitable cell lines in a study once every c. 125 studies
- No cell lines showed changes in both product quality and productivity

Suitability

- Factors known to cause loss of productivity by GS cell lines
 - Subculture protocols
 - Medium composition
- In some instances, loss of stability can be slowed or reversed by changing subculture protocol or media compositions

Acknowledgements

- Mike Davies, Analytical Development
- The people in Lonza Biologics, Slough who generated these data over the last 10 years
 - Cell Culture Process Development
 - Analytical Development
 - Purification Development