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GS Gene Expression System:

GS, biochemical comparability, and cell line stability

Structure of Workshop

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- Updates on:
 - Process development and biochemical comparability of an antibody from GS cell lines
 - Stability of GS cell lines



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Process development and biochemical comparability of antibody from GS cell lines

Objective of study

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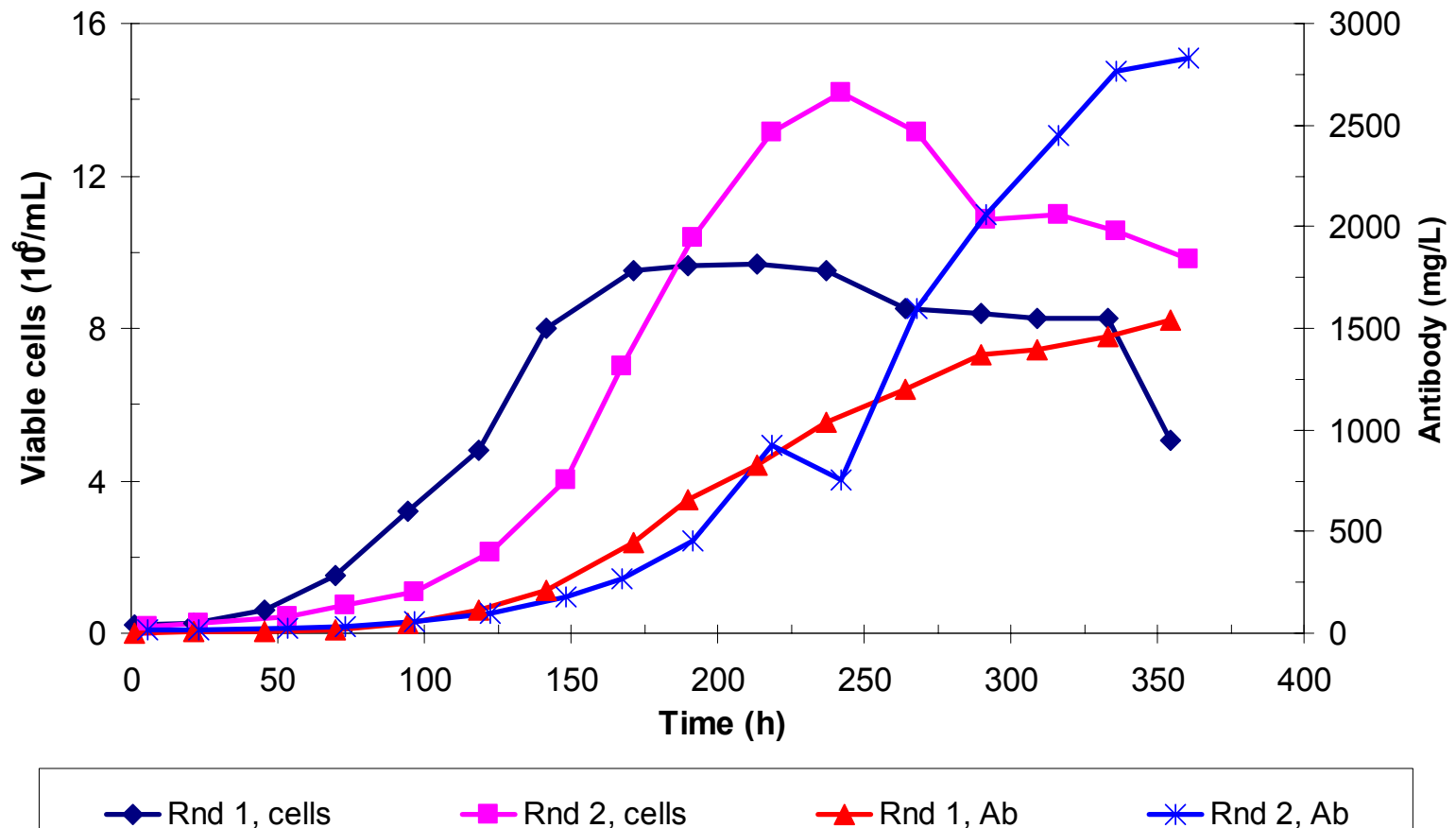
- Demonstrate biochemical comparability of antibody after process development has given substantial increases in volumetric productivity
 - Successive iterations of process development improved both cell growth and specific productivity

- GS-CHO and GS-NS0 cell line
- Fed-batch, bioreactor cultures
- Chemically-defined, animal component-free and protein-free cell (CDACF&PF) culture process

- Biochemical analysis
 - SDS-PAGE (reduced and non-reduced)
 - IEF
 - Antibody fragmentation by Western blotting
 - Tryptic mapping
 - Oligosaccharide profiling by MALDI-TOF MS
 - Isoform profiling by electrospray ionisation MS

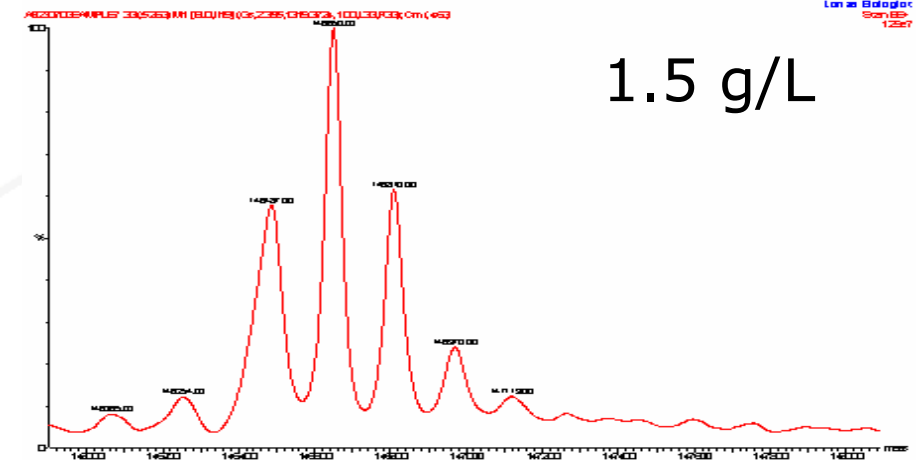
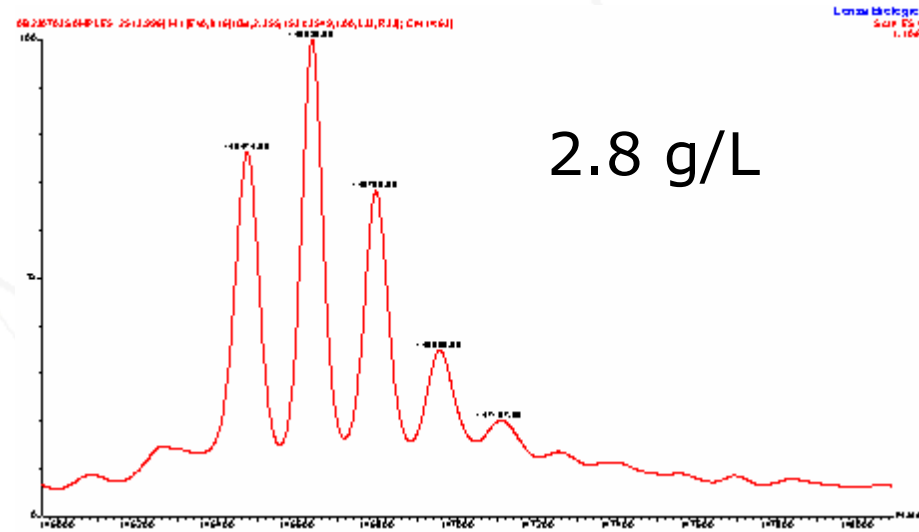
GS-CHO cell line grown in fed-batch, bioreactor culture: two rounds of process development

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ESI-MS analysis of antibody produced by a GS-CHO cell line

- A range of isoforms were detected in both batches
- Isoform distribution was comparable between the two process development iterations
- Isoforms were consistent with detected range of oligosaccharide structures (data not shown)



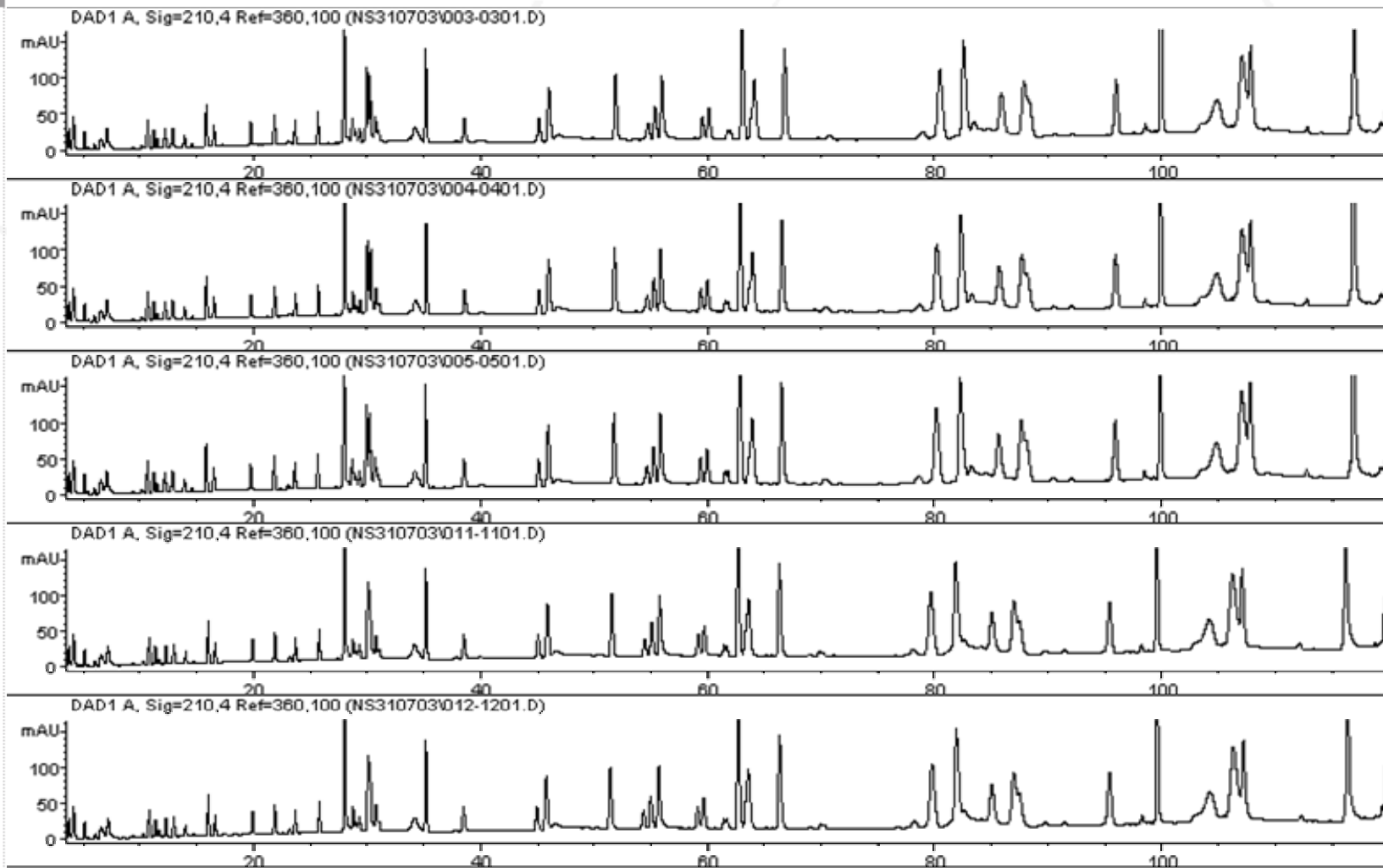
Biochemical comparability of antibody from 1.5 and 2.8 g/L GS-CHO bioreactor cultures

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Analysis method	Comparable?
Reduced and non-reduced SDS-PAGE	Yes
IEF	Yes
IgG fragments by Western blot	Yes
ESI-MS	Yes, consistent with expected distribution and with glycosylation profile
MALDI-TOF MS	Yes, consistent with expected profile
RP-HPLC tryptic peptide map	Yes

RP-HPLC tryptic peptide maps of Ab produced by a GS-NS0 cell line during process development

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Biochemical comparability of antibody from GS-NS0 bioreactor cultures

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Analysis method	Comparability?
Reduced and non-reduced SDS-PAGE	Yes
IEF	Yes
IgG fragments by Western blot	Yes
ESI-MS	Yes, consistent with expected distribution and with glycosylation profile
MALDI-TOF MS	Yes
RP-HPLC tryptic peptide map	Yes

Process development and biochemical comparability of antibody from GS cell lines

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- Antibody from CDACF&PF bioreactor processes for GS cell lines was extensively characterised
- GS-CHO
 - Antibody was biochemically comparable after
 - Volumetric productivity increased from 1.5 to 2.8 g/L and specific productivity increased from 0.9 to 1.2 pg/(cell·h)
- GS-NS0
 - Antibody was biochemically comparable after:
 - Volumetric productivity increased from 0.37 to 1.4 g/L and specific productivity increased from 0.5 to 1 pg/(cell·h)

The slide features a white background with three large, overlapping, light gray circles. A horizontal gray bar spans the width of the slide, intersecting the circles. On the left side of this bar, there is a small, colorful strip of images showing laboratory equipment and biological samples. The word 'Lonza' is printed in a bold, black, sans-serif font in the upper right corner.

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Antibody consistency with generation number

Stability of GS cell lines - FAQs

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- What proportion of GS cell lines are unstable?
- Have you seen changes in biochemical comparability with increasing generation number?
- What can cause cell line instability?

What do we define as “cell line stability”?

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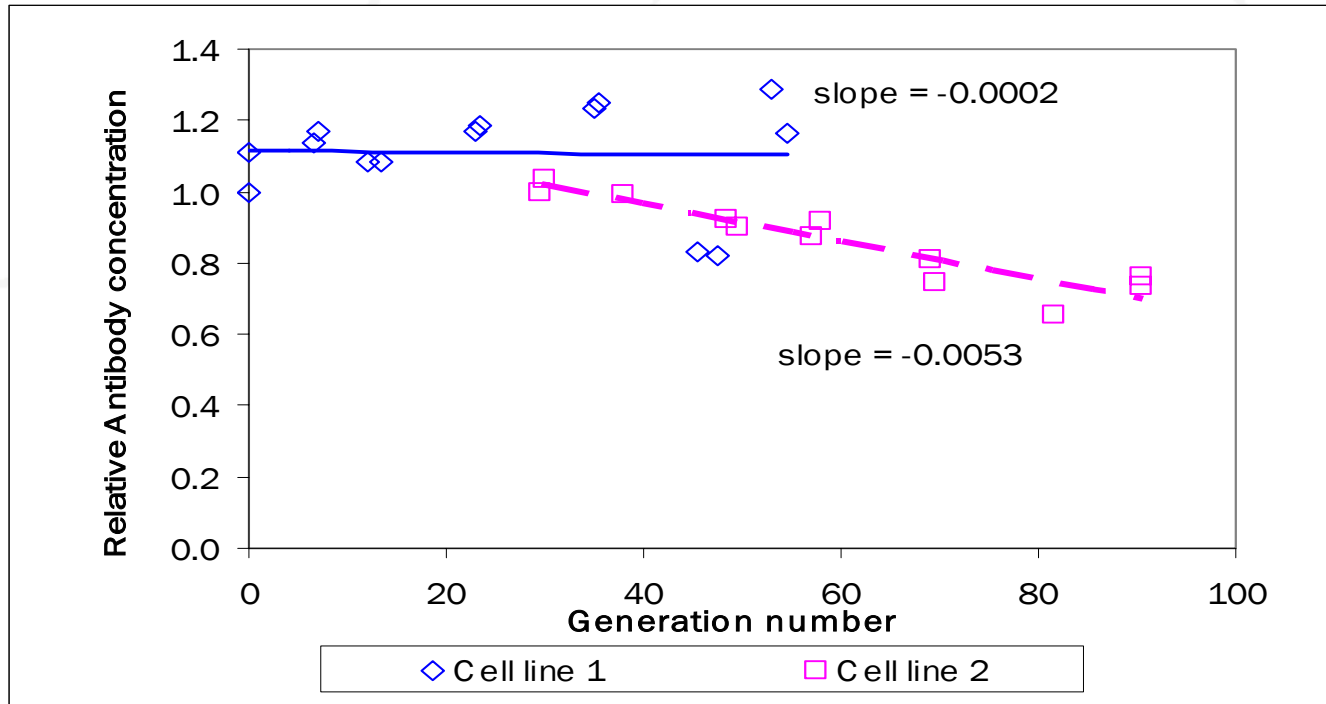
- Define cell line stability as:
 - Consistency of a given growth or productivity parameter, or the product’s characteristics with increasing generation number
- Why “consistency”?
 - Stability of the cell line with increasing generation number contributes to overall process consistency

Stability of GS cell lines

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- More specific definition of a stable cell line:
 - A cell line that does not show a change in either quality and quantity of the recombinant protein *across the manufacturing window*
- How can this be tested?
 - Trend changes in cell culture parameters with increasing generation number in a good scale-down model of the inoculum and production cell culture processes for required number of generations
 - Analyse purified product from different points in the study

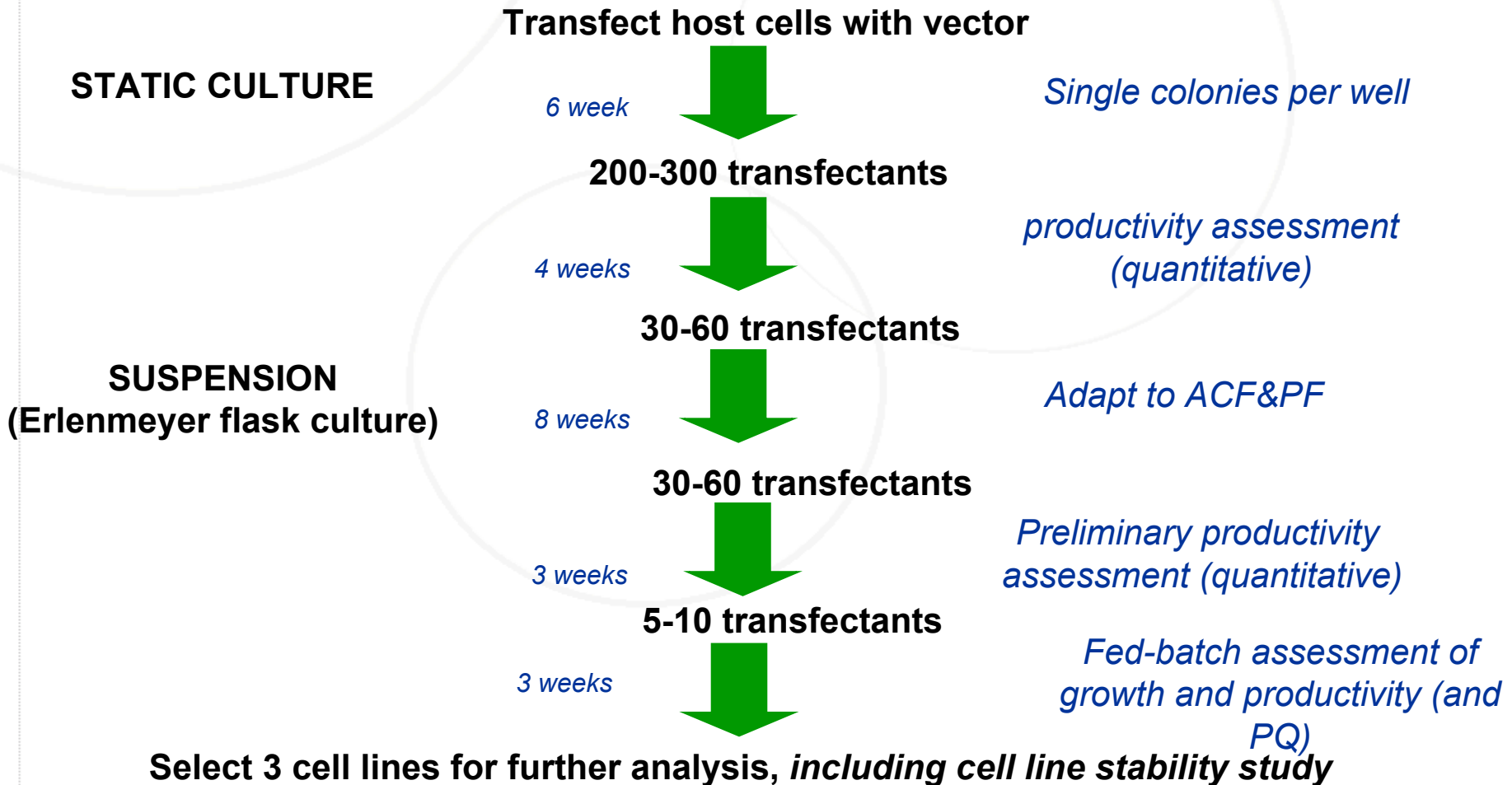
Trending changes in cell growth and productivity parameters



- Fit line to parameter data by least squares method
- Is there a substantial change in the parameter value across the manufacturing window?

Selecting high producing cell lines: strategy selects against grossly unstable cell lines

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- Data from randomly selected GS-NS0 cell line development programmes undertaken over the last 10 years
 - 21 antibody products
 - 52 different cell lines
 - Data analysed by testing against the definition of an unstable cell line:
 - Product *must* be biochemically comparable at the start and finish of the study
 - does the change in antibody concentration across the 40 generation manufacturing window exceed 30%? (internal Lonza guidance)

Have you seen changes in biochemical comparability with increasing generation number?

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- Data available from 38/52 cell lines
- Changes seen for 2/38 (3%) cell lines
 - equates to 1/21 antibodies
 - In each instance, there was an alternative cell line available that was used instead
- Cell lines exhibiting changes in product quality with increasing generation number are infrequent

What proportion of GS cell lines are unstable?



Change >30% seen in	Proportion
Antibody concentration	0.23 (12/52)
Both antibody concentration and specific production rate	0.18 (9/49)
Specific production rate, but not antibody concentration	0.10 (5/49)

- Main cause of variation in antibody concentration is change in specific production rate
- Also see changes in cell growth: these may have a beneficial or deleterious impact upon stability of antibody concentration

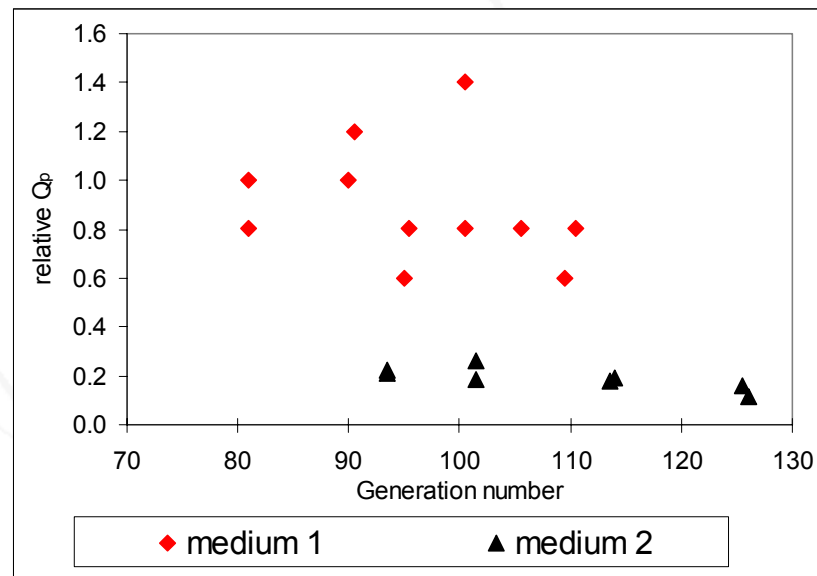
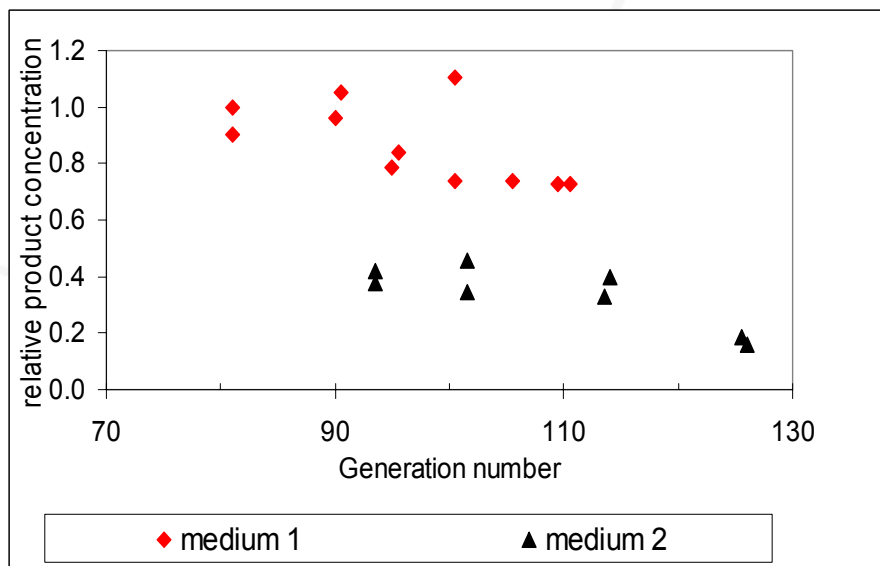
Impact of subculture day upon behaviour of a GS-NS0 cell line

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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 for about 35 generations with different subculture regimes and then "overgrown"
- 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)
- All cultures overgrown in medium 2
- Combination of endogenous GS activity and medium supplements by-passed MSX selection resulting in decline in specific productivity

- Biochemical comparability
 - Antibody produced in cell culture processes with increased specific and volumetric productivities was comparable to the original process

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- A horizontal decorative bar is positioned below the title and logo. It features a series of small, colorful images related to biotechnology, including what appears to be a microscope, test tubes, and laboratory equipment.
- Stability of GS cell lines
 - In-house data suggests that cell lines exhibiting changes in product quality occur infrequently
 - About 77% of GS-NS0 cell lines were stable
 - Factors known to cause instability of GS cell lines:
 - subculture protocols
 - medium composition
 - Loss of stability can be slowed or reversed by changing subculture protocol