

Stability Of GS-NS0 Cell Lines

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Introduction

To support approval of a drug for therapeutic use, the regulatory guidance recommends that one of the studies tests for consistent production of the intended product of interest (1). For a manufacturing process using mammalian cells, the cells are serially cultured to generate the inoculum for the production bioreactor. In a fed-batch process, product taken from two points in the inoculum expansion phase (at the beginning and at the limit of *in vitro* cell age) is used to assess whether the product changes with increasing generation number. Additionally, it is important to understand how the productivity of the cell line changes with increasing generation number.

Lonza Biologics' Glutamine Synthetase (GS) Gene Expression System and the DHFR-CHO expression system are both used to express recombinant antibodies from mammalian cells. Although there is a large body of work in the public domain describing stability studies on DHFR-CHO cell lines, there are only a limited number of studies describing the stability of cell lines generated using the GS system. This presentation describes some of Lonza's experience with the stability of recombinant-antibody producing GS-NS0 cell lines.

What is cell line stability?

- Cell line stability can be defined as:
 - Consistency of a given growth or productivity parameter, or the antibody's characteristics with increasing generation number.
- A stable cell line does not show a change in either quality or quantity of the recombinant antibody across the manufacturing window.

Why is cell line stability important?

- The key importance of cell line stability comes from its linkage to the biochemical consistency of the antibody and to process consistency, which are both linked to drug safety and efficacy.
- Cell line stability is also linked to process robustness and to process economics.

Results and Discussion

How Can Cell Line Stability Be Assessed?

In order to assess the suitability of a cell line for the manufacture of a recombinant antibody, Lonza Biologics routinely tests the 'stability' of cell lines. This is accomplished by trending changes in growth and productivity characteristics (viable cell concentration, specific production rate, etc.) of the cell line for the required number of generations in a scale-down model of the inoculum and production cell culture processes. Each cell line is serially sub-cultured for a number of generations that includes the manufacturing window and, at about 10 generation intervals, cells are assessed in a scale-down, Erlenmeyer flask model of the production bioreactor process. Purified product from different points in this study is also analysed.

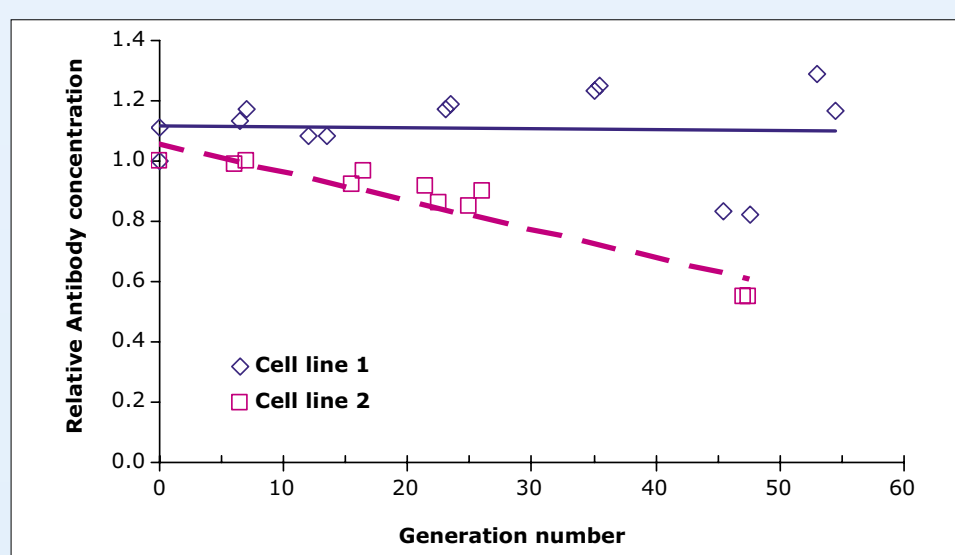
Over the review period, the techniques used to detect changes in product quality have changed as new methods were introduced. Currently, the techniques used for antibody analysis include peptide mapping, MALDI-TOF-MS and ESI-MS. The techniques that are common to all the studies reviewed are SDS-PAGE and IEF electrophoresis of Protein A purified antibody.

Analysis was done against the following definition of a stable cell line:

- The product must be biochemically comparable, using the analytical techniques available, at the beginning and end of the study.
 - For the electrophoretic techniques, the antibody from the start and end of the stability study had to give similar banding patterns after electrophoretic separation.
- The change in antibody concentration across the 40 generation manufacturing window is less than 30%.
 - A value of 30% was chosen as this is twice the expected coefficient of variation for the ELISA assays used in some of the early programmes.

Data from a stable and an unstable GS-NS0 cell line are shown in Figure 1: cells were serially sub-cultured for about 45 to 55 generations. In order to trend changes, lines were fitted to the parameter data by the least squares method. The antibody concentration for cell line 1 changed by only 1% over the period of the study. Cell line 2 did exhibit a substantial change (36%) and is considered to be unstable with respect to antibody production.

Figure 1. Antibody concentration data for a stable and an unstable GS-NS0 cell line are used to illustrate the changes that can be seen when trending the behaviour of different cell lines.



References

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- Coco-Martin JM, Oberink, JW, Brunnik F, et al. (1992). Hybridoma 11:653-665.
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- Barnes LM, Bentley CM, Dickson AJ (2004) Biotechnol Bioeng 85:115-121.

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- The data presented in this poster were obtained from a randomly selected sub-set of cell line development programmes, covering 23 antibodies, undertaken at Lonza Biologics over the last 10 years.
- Data were generated using cell lines adapted to either serum-free or protein-free media.
- None of the cell lines included in this review had been amplified.
- Selection pressure for the recombinant phenotype was maintained through use of glutamine-free media: MSX was not included in the media.

Table 1. Occurrence of changes in the biochemistry of antibodies upon extended sub-culture of GS-NS0 cell lines

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

Table 2. Summary of studies investigating the change in antibody concentration and specific production rate (Qp) for GS-NS0 cell lines with increasing generation number

Condition	Proportion
No change in antibody concentration	0.79 (48/61)*
No change in either antibody concentration or Qp	0.69 (40/58)**
>30% change in Qp only	0.09 (5/58)
>30% change in antibody concentration only	0.05 (3/58)
>30% change in both antibody concentration and Qp	0.17 (10/58)

- * antibody concentration data available from 61 cell lines
- ** antibody data together with Qp data only available for 58 cell lines

- The data presented in Tables 1 and 2 show that:
 - Cell lines exhibiting changes in product quality are infrequent - for each programme where this occurred, there was an alternative cell line available that could be used to manufacture the drug substance. Although there are reports (2) of changes in antibody produced by hybridomas with increasing generation number, the authors are not aware of similar published data for recombinant antibodies.
 - GS-NS0 cell lines meeting the antibody concentration criterion for a stable cell line occur at about 79% of the time.
 - Further examination of the data showed that a large proportion of all the cell lines (69%) were stable for both cell growth and the specific production rate (Qp). This is not surprising as antibody concentration is a function of both Qp and the time integral of the viable cell concentration. Although changes in Qp were seen in about 9% of the cell lines, there were concomitant changes in cell growth such that there was no overall effect upon the antibody concentration.
 - A change in antibody concentration was correlated with a change in Qp for about 17% of cell lines.

- The definition used in this study for a stable cell line contains terms for both product quality and a productivity parameter. The data (Tables 1 and 2) show that, for GS-NS0 cell lines, if the individual parts of the definition are used to assess the stability of the cell line, a cell line is about 13-times more likely to be classified as unstable due to the change in antibody concentration being too large than because of a change in product quality.

- The question is then, what is the frequency that a cell line is classified as stable using the complete definition?

- The complete data set is only available for 46 cell lines. About 78% (36/46) of the GS-NS0 cell lines studied were stable, as they did not exhibit a change in product quality nor did they exhibit a substantial change in antibody concentration.

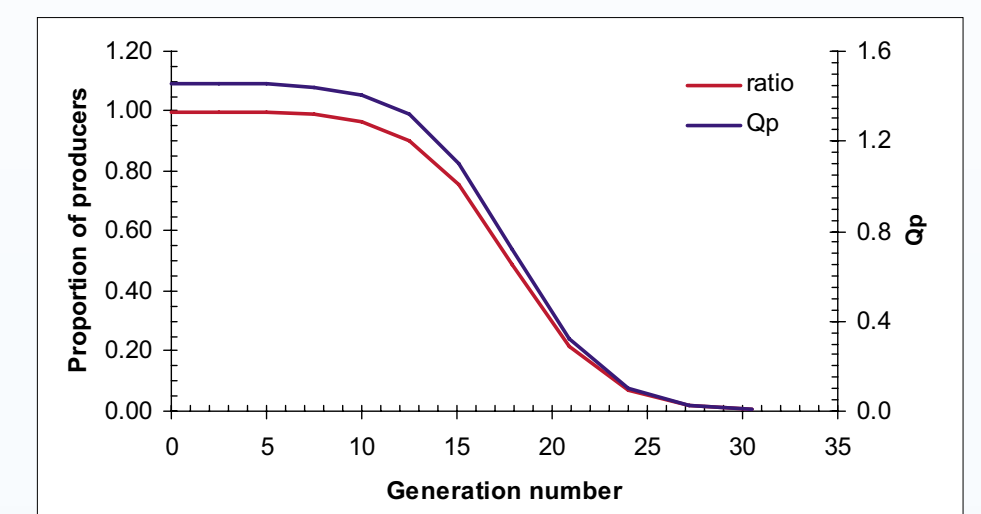
Modelling The Outgrowth Of A Lower Producing Sub-population

- Hypothesise that antibody production imposes a metabolic burden upon the cell that may lead to a reduction in the growth rate. Thus, cell lines with a higher Qp should grow more slowly than cell lines with a lower Qp.
 - For GS-NS0 cell lines, differences in growth rate that correlate with antibody productivity have been seen (3).
- If it is further hypothesised that a sub-population with a lower Qp (and higher growth rate) appears in the cell line, it can be shown mathematically that the faster growing sub-population will eventually dominate the population (Figures 2 and 3). Consequently, the measured Qp and antibody concentrations obtained with the cell line will change with increasing generation number (Figures 2 and 3).
 - Phenotypic variation with respect to growth rate and productivity can be demonstrated within a cell line.
- If this hypothesis is true, there should be agreement in the shape of the curves between a model where a small, faster growing (i.e. less productive) population appears in the producer population, and for the observed data.

Summary (1)

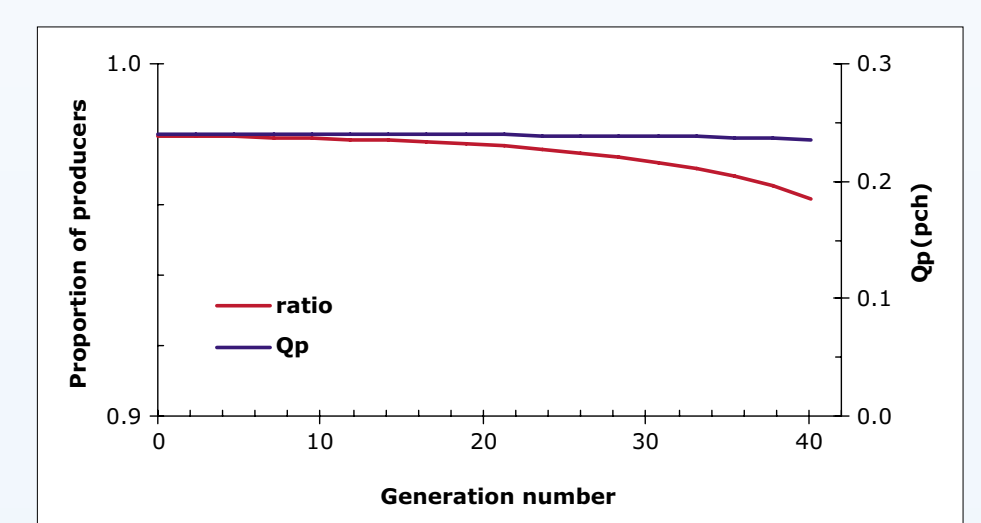
- Stability of GS-NS0 cell lines
 - Data generated by Lonza Biologics over the last 10 years suggests that cell lines exhibiting changes in product quality occur infrequently.
 - About 79% of GS-NS0 cell lines were stable, showing <30% change in antibody concentration across the manufacturing window.

Figure 2. Modelling the extreme situation: non-producer sub-population introduced into the producer cell line



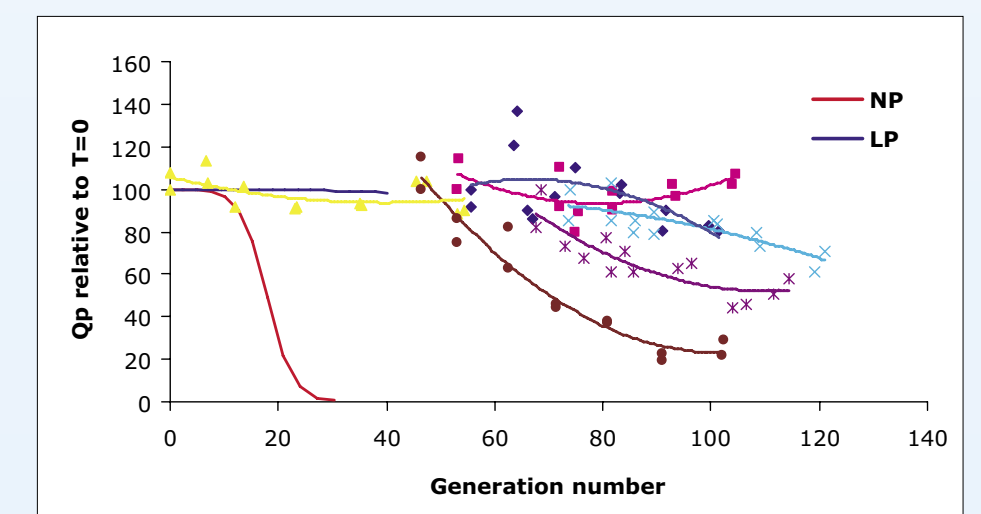
- Small sub-population (0.05%) of non-producers introduced at the start of the stability study
 - Difference in $\mu = 0.003 \text{ h}^{-1}$
- By generation 30, the model shows that the population has been taken over by the non-producer and the measured Qp has fallen to zero.

Figure 3. Modelling the more likely scenario: a low-producer sub-population introduced into the producer cell line



- Small sub-population (0.05%) of lower-producers introduced before the start of the stability study
 - Difference in $\mu = 0.001 \text{ h}^{-1}$; difference in Qp = 1.2 pg cell⁻¹ h⁻¹
- By generation 30, the model shows that the Qp and the proportion of low producers have changed slightly.
- Eventually, the model predicts that the low producing sub-population will take over the population (not shown).

Figure 4. How good is the growth rate difference model? Curves for non-producer (NP) and low producer (LP) populations in Figures 2 and 3 are plotted against a randomly chosen panel of stable and unstable GS-NS0 cell lines. To improve clarity, the curves for the NP and LP models, and a stable GS-NS0 cell line are off-set from the other data.



- The agreement between the model and experimental data is poor, as the shapes of the curves for the experimental data are very dissimilar to the curves generated by the model (Figure 4). Thus, the hypothesis that the change in the productivity characteristics with generation number is the result of the outgrowth of a faster growing, lower producing sub-population is not supported.
- The loss of productivity from GS-NS0 cell lines, at least under the conditions used at Lonza Biologics, is probably not due to the appearance of a faster growing sub-population.
- An alternative hypothesis (4) to explain the changes in productivity of GS-NS0 cell lines with increasing generation number, relates the levels of LC and HC mRNA to a putative saturation point for utilisation of mRNA in translational / secretory events.

Summary (2)

- When changes in specific productivity with increasing generation number are modelled for populations containing varying proportions of high and low producers with different growth rates, the modelled decline in specific productivity does not match the observed data.
- The loss of productivity from GS-NS0 cell lines is probably not due to the appearance of a faster growing sub-population.