

Development of Robust and Scalable Processes

Designing in 'Manufacturability' at an Early Stage

Andrew Racher
Lonza Biologics

Clinical Trial Supply for Biologics,
Lisbon, October 2005

Structure of Talk

1. Introduction
2. Technology Platforms – USP Processes
3. Technology Platforms – DSP Processes
4. Summary

Introduction

Time – The Faster the Better

- Industry is facing ever shorter development timelines for supplying material for clinical trials

- Short timelines need:
 - Rapid USP and DSP development strategies
 - Strategies that have high probability of success
 - Minimal development work
 - Easily scaleable, robust processes
 - Still meet other criteria, for example:
 - ◆ Economic, e.g. minimum productivity needed to supply clinical trials
 - ◆ Regulatory, e.g. consistent product quality
 - ◆ Safety, e.g. acceptable virus clearance

Speed in Development - USP

- Cell line construction and selection often the slowest step
 - Need to complete as rapidly as possible, ideally without compromising productivity or quality criteria

- Use of platform technology
 - Culture media, feeds, growth conditions, etc.

- Predictive scale up systems
 - Challenge is to predict manufacturing behaviour of cell lines at very early stage and select clones with, e.g., appropriate growth characteristics

Speed in Development - DSP

- Biophysical characteristics (e.g. pI, solubility) are inherent to molecule
 - Dictate values of parameters and therefore protocols used

- Use of platform technology
 - Resins, regeneration conditions, flow rates, etc.

- Predictive scale up systems
 - Challenge is to develop purification process that fits with plant capabilities and gives acceptable purities, yields, etc.

Process Development: USP vs. DSP

- Cell lines for antibody production
 - Cell line characteristics are selectable
 - ◆ Select cell line to fit standard USP process

- Antibody
 - Characteristics are inherent to molecule
 - ◆ Select DSP process to fit molecule
 - Situation changing

Technology Platforms

USP Processes

USP Development Strategy

- Goal to create stable, high producing cell lines
 - Grow in suspension culture
 - Grow in a chemically defined, animal component-free (CDACF) medium

- High producing cell lines result from:
 - Host cell
 - Expression system
 - Transfection and selection protocol
 - Rapid creation – no gene amplification

- High productivity processes result from:
 - Media and feeds
 - Optimised inoculum and manufacturing process

- Choose high producing cell lines that fit manufacturing process by selecting cell lines in good scale-down model of USP production process

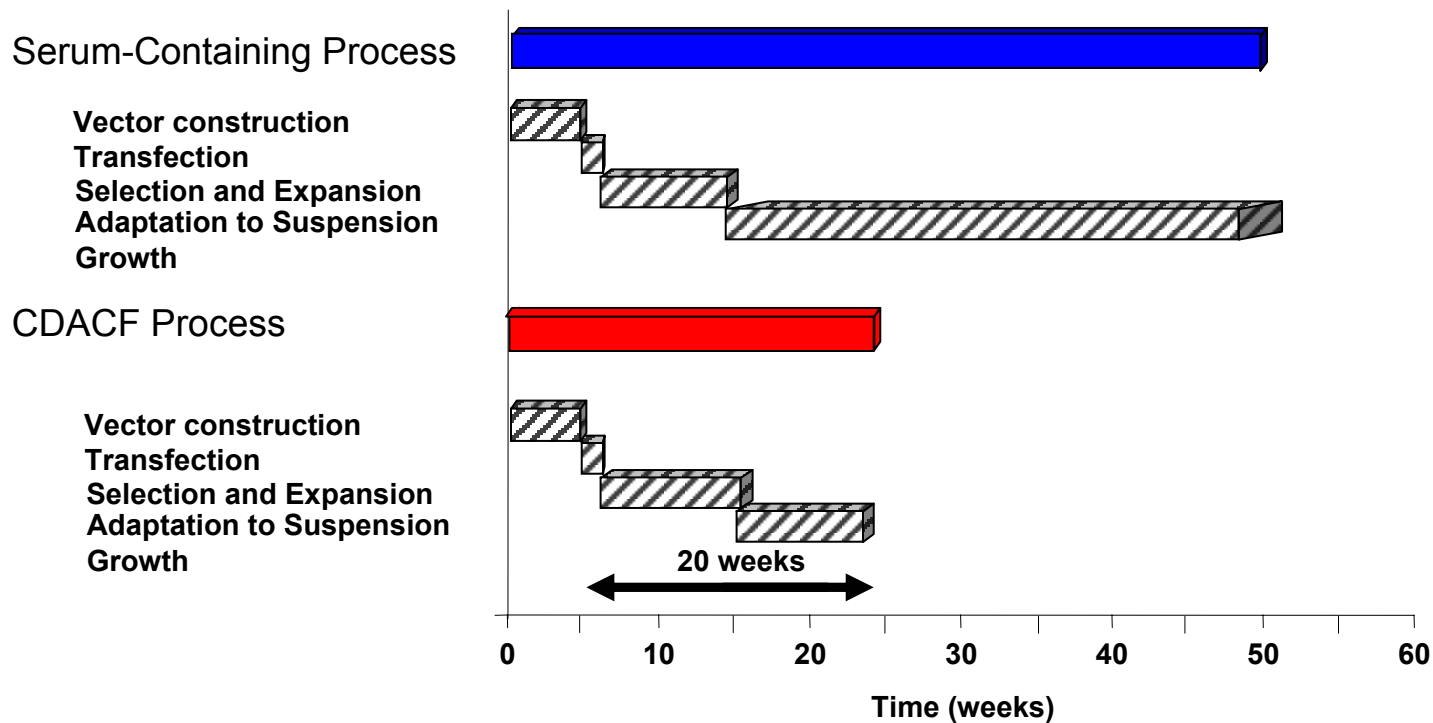
Faster Cell Line Construction and Selection: A better host cell

- Adaptation from selection medium to suspension growth in production medium is often longest stage
 - Use pre-adapted host cell line
 - ◆ CHO vs. NS0, Sp2/0 cell lines

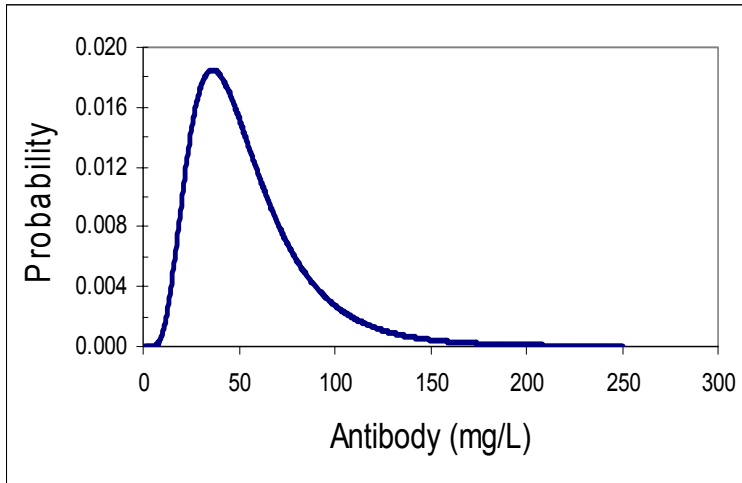
- Developed CHOK1SV (suspension variant)
 - Grows as single cell suspension
 - Pre-adapted to growth in CDACF media
 - Exhibits good growth characteristics
 - ◆ Reach high maximum viable cell concentration
 - ◆ Able to maintain cultures at high culture viability

Timeline Reduction with CHOK1SV

- Use of suspension variant of CHO-K1 pre-adapted to growth in CDACF media reduced time taken to generate cell lines by 6 months

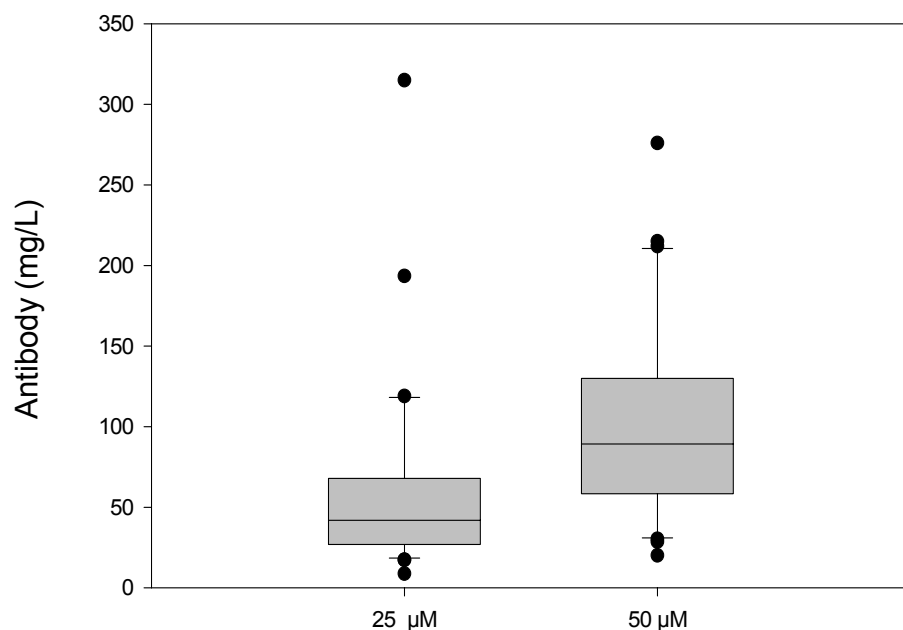


Cell Line Selection: High producers are infrequent



- Transfection method that generates large numbers of stable transfectants
 - maximise the range of productivities
 - ◆ *No. of cell lines = p(good cell line) * no. screened*
- Stringent selection to eliminate lower producers
- High throughput methods, e.g. FACS + cell surface product capture, to screen large numbers efficiently
- Combination of above

Cell Line Selection: Selection stringency



Selection conditions - MSX concentration

- Influence of selection conditions for GS-CHO cell lines transfected with cB72.3 antibody
- More stringent selection increased median concentration and changed location of distribution
- Cell lines were not amplified

Finding High Producing Cell Lines (1)

- Screening system should be designed with reference to the final production process
- Predictive scale up systems – challenge is to predict manufacturing behaviour of cell lines at very early stage and select cell lines with e.g. appropriate growth characteristics

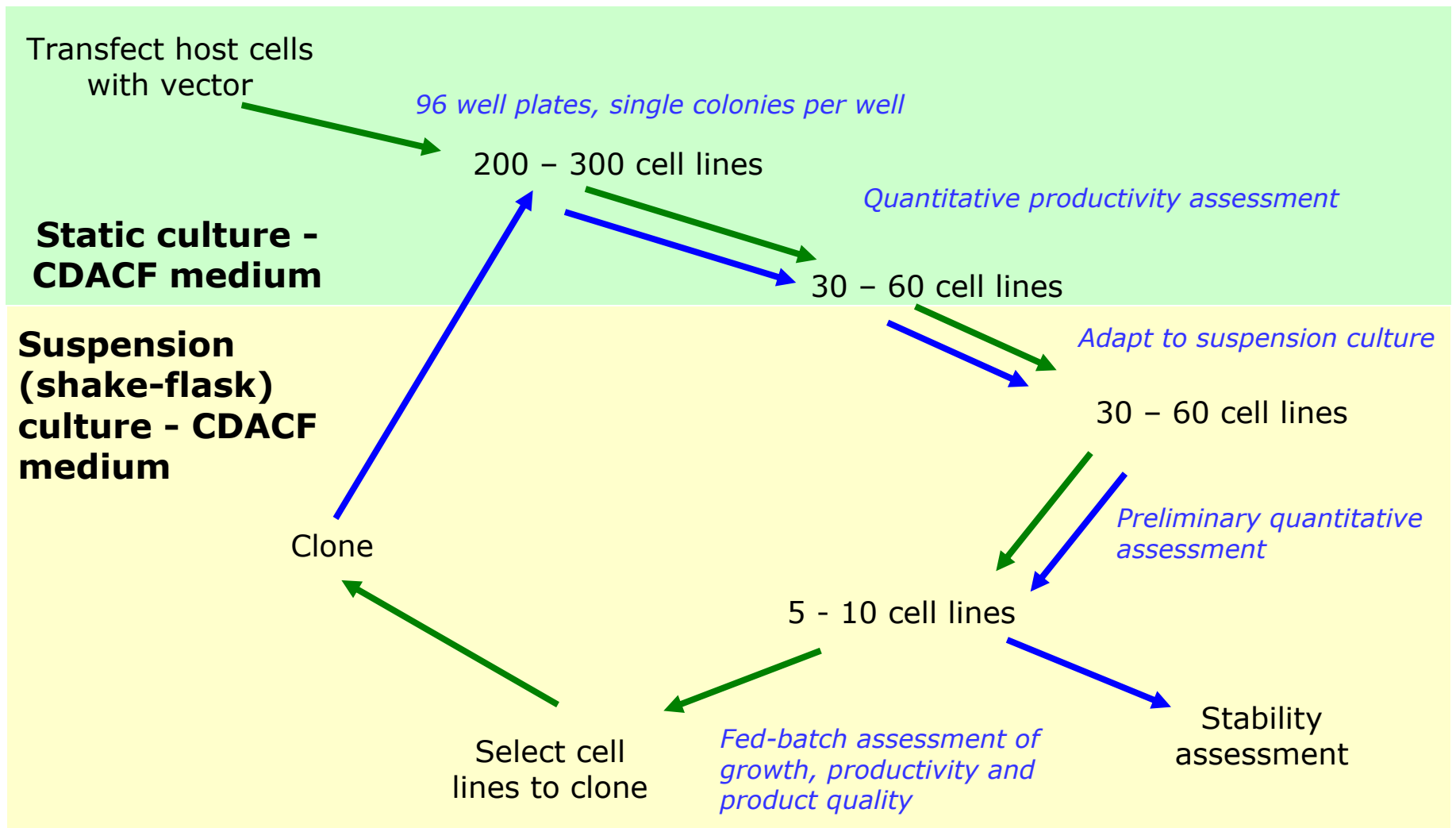
Finding High Producing Cell Lines (2): USP process outline

- Outline of USP process provides criteria for selecting production cell lines

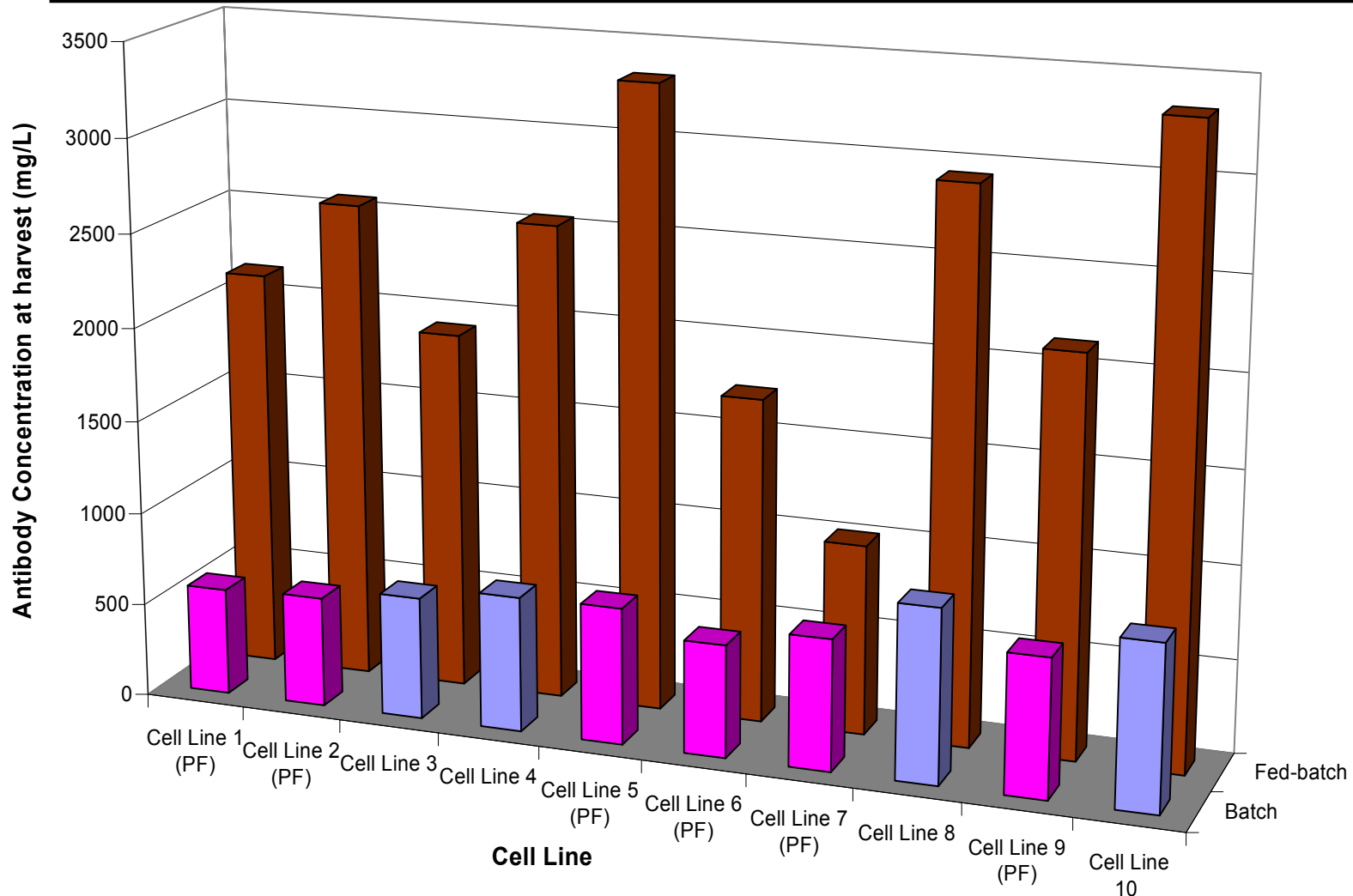
- Inoculum process
 - CDACF medium
 - Fixed subculture interval
 - Minimum value for culture dilution at subculture

- Production process
 - CDACF medium
 - Fed-batch
 - Upper limit to process duration

Finding High Producing Cell Lines (3)

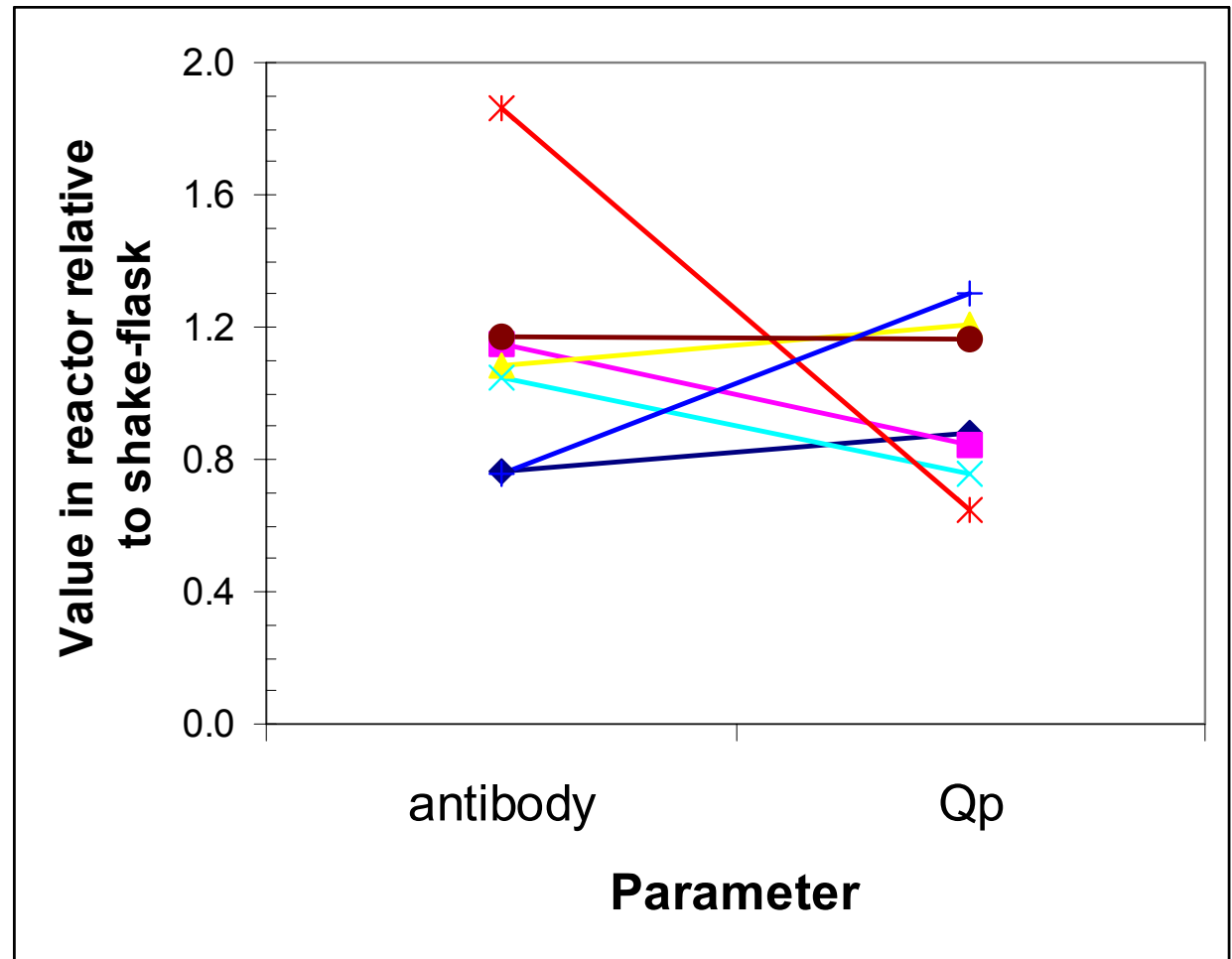


Batch and Fed-Batch Culture: Comparison of cell lines generated using serum-containing and CDACF media



Fed-batch shake-flask vs. fed-batch bioreactor

- Relationship between productivity characteristics of the lead cell lines making seven different antibodies made in GS-NS0 cell lines



USP Process Development

- Rapid USP development
 - E.g. pre-adapted host cell line

- Easily scaleable, robust processes
 - Screen in good scale-down model
 - ◆ Eliminates cell lines that do not have suitable growth characteristics

- High probability of success and minimal development work
 - Screen in scale-down model with good predictive power

- Product quality
 - Screen
 - Engineered cell lines (Glycart, Biowa)

Technology Platforms

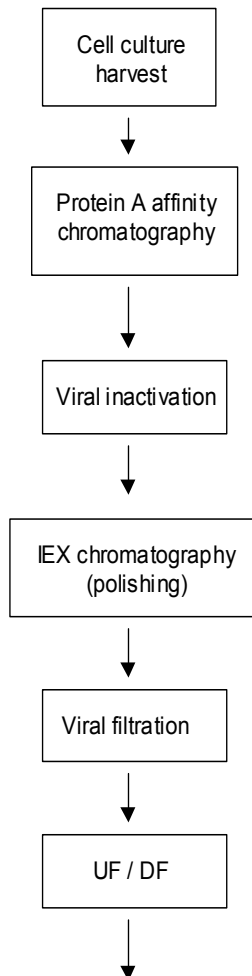
DSP Processes

Antibody Purification (1)

- All antibodies are not the same
 - Variation in allotype and CDR sequences can contribute to differences in biophysical characteristics

- Characteristics (e.g. pI) are inherent to molecule
 - Select DSP process to fit molecule
 - Platform process not standard process
 - ◆ Antibody solubility, sensitivity to pH transitions, propensity to form aggregates, etc all lead to changes in DSP process

Antibody Purification (2)



- Protein A affinity chromatography step is core of platform DSP process
 - Highly selective interaction with Fc region
 - High yield and purity in single step
 - Most antibodies behave in similar fashion
 - ◆ focus on limited range of parameters

Antibody Purification (3)

■ Aggregation

- Conventionally trade aggregate content vs. product loss during aggregate removal
- Harvest early – trade off product loss vs. need to clear aggregates
- Altering molecule's characteristics *
 - ◆ Modified antibody by directed evolution
 - ◆ Increased yield on Pn A chromatography, increased pH stability, increase solubility, decrease soluble aggregates

* Borneman, Antibody Production & DSP Meeting, San Diego, '05

Platform DSP Process

- Key benefits of platform process
 - Leverage historical experience with antibodies
 - Reduce project cycle times
 - Improve efficiency of resource utilisation and ease of implementation in manufacturing
 - Use of generic validation data

Leveraging Historical Experience: Contaminant clearance

- Platform DSP process
 - Large data set generated by repeated utilisation
 - Know what log-reduction to expect for a range of contaminants and impurities e.g. HCPs, viruses, DNA, endotoxins, etc.
 - ◆ High probability of success
 - ◆ Still have to confirm virus reduction experimentally for regulatory submission

Reducing Project Cycle Times: Faster development processes

- Develop DSP process *de novo* - >> 100 parameters
- Main variables = pI, solubility (aggregate levels, solvation)
- Use platform process - focus on 10% of process parameters that need to be varied between molecules
 - Elution buffers for IEX
 - Straight forward when have panel of buffers and conditions for different pI antibodies

Efficient Resource Utilisation and Ease of Transfer

- Standardisation
 - Focus development effort on non-standard parameters
 - ◆ E.g. binding capacity, elution buffer, etc.
- Plant throughput
 - For a CMO, maximise number of batches and therefore revenue
 - Experimental focus becomes ,e.g., not which buffer to use but but how can I minimise number of cycles for given unit operation?
- Ease of Transfer
 - Manufacturing plant staff are not having to be trained on a bespoke DSP process for each new product

Designing in 'Manufacturability'

- Know the limitations of your plant
 - if I develop a DSP process, does manufacturing plant have sufficient tank volume to cope with buffer volumes needed?
 - ◆ Elute in small or large volume?
 - Effluent disposal e.g. detergent containing buffers
 - ◆ What are local regulatory requirements?
 - ◆ Detergent virus inactivation step done at low or high product concentration

Documentation and Quality

■ Purity

- Product specification at phase I: range of acceptable values for characteristics are fairly wide
- Use generic cf. product specific assays

■ Documentation

- Template documents
 - ◆ Documents are still product-specific, but format and most parameter values do not change
- *De novo*, multiple tens of documents will need to be written in full, exact number plant dependent

SUMMARY

Designing in 'Manufacturability' at an Early Stage

Summary

- Ever shorter development times to get material for clinical trials - 'first time success'
- Platform USP
 - Select cell line to fit USP process
- Platform DSP
 - Use experience and historical data to leverage process development
- Use of well-characterised platform technologies and appropriate cell line selection criteria help eliminate difficulties during process development
- Platform processes minimise development work for in-market supply
 - optimising rather than re-developing

Acknowledgements

- Dr Julian Bonnerjea
- Cell Culture Process Development Group,
LB, Slough
- Assay Development Group,
LB, Slough