

The Waterside Conference
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Lonza

**Rapid Production of Antibodies by
GS-CHO Pools**

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Overview

- Introduction
- Aim
- Overview of pooled transfection procedure
 - Time taken
 - Culture volumes
- Growth and productivity of GS-CHO pools in disposable bioreactor
- Reproducibility
- Duration of model monoclonal antibody expression
- Product quality
- Strategies for scale up
- Comparison with PEI-mediated transient gene expression in CHO cells
- Summary

Introduction

- Cell line development is a critical path activity that limits the availability of material for starting pre-clinical studies
 - e.g. formulation evaluation or assessment of functional, pharmacokinetic or immunological properties.

- Several candidate therapeutics assessed before choosing one for cell line development
 - Host cell specific differences in antibody integrity

- The availability of antibody produced in the same host cell and cell culture process as the final cGMP manufacturing process has the potential to reduce a programme's duration.

Accelerating Supply of Material

- Vector design
 - e.g. Inclusion of matrix association regions (MAR) elements

- Use of host cells for genetic manipulation that have been pre-selected to grow under manufacturing conditions (e.g. suspension culture in chemically-defined, animal component-free (CDACF) medium)
 - e.g. CHOK1SV

- Use transient expression or non-clonal cell line/pool for early development studies

Antibody Expression Formats

Transient



1 - 10's of mg/L
Days to weeks



Stable



1 - 4 g/L
> 4 months

Aim

- To develop a generic, CDACF process for the rapid production of small quantities (5 to 50 g) of antibodies in CHO cells.

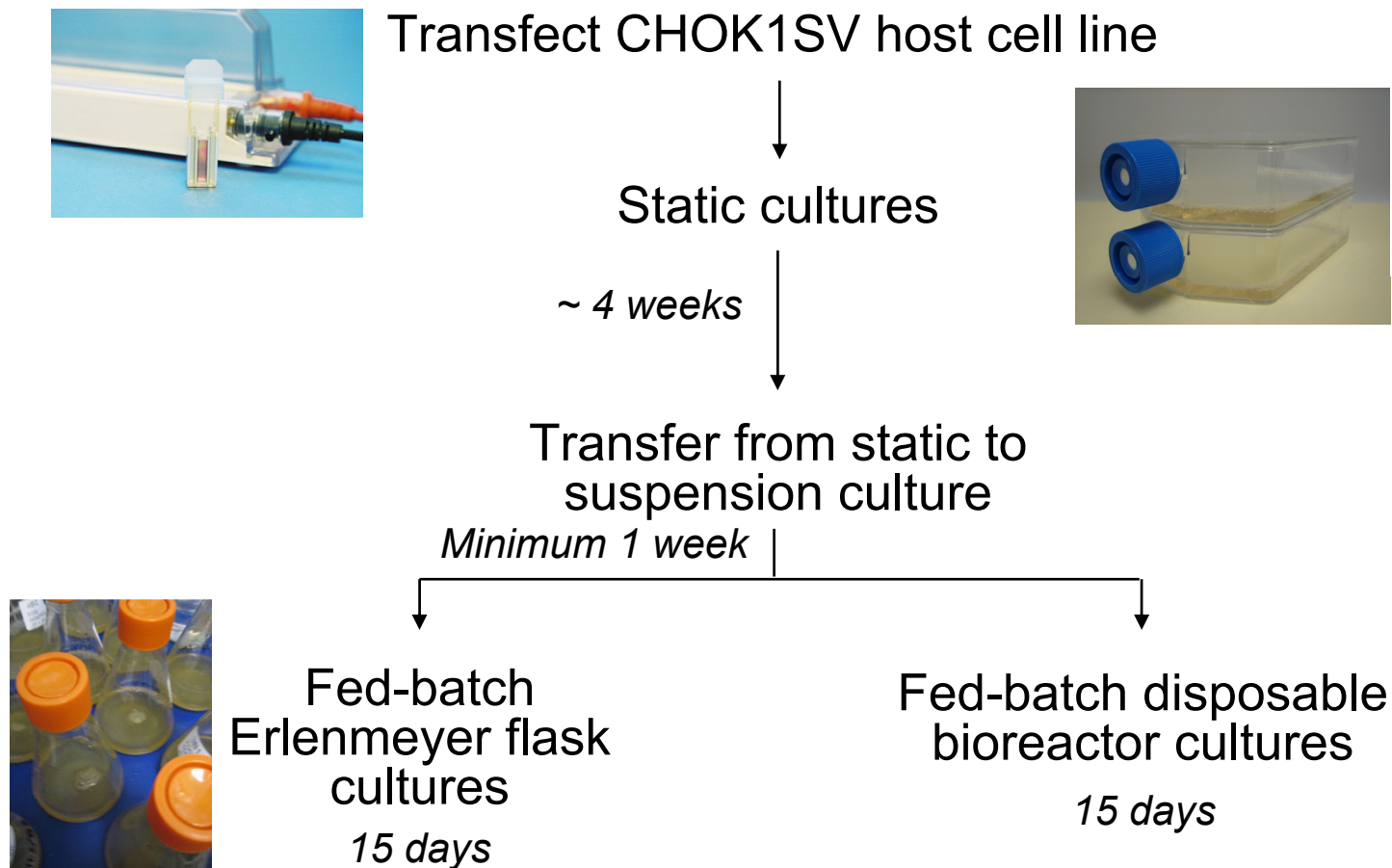
How can we combine harvest concentrations of stable system with speed of transient system?

- CHOK1SV host cell line
 - Grow in suspension culture in CDACF medium

- GS expression vector expressing model antibody cB72.3

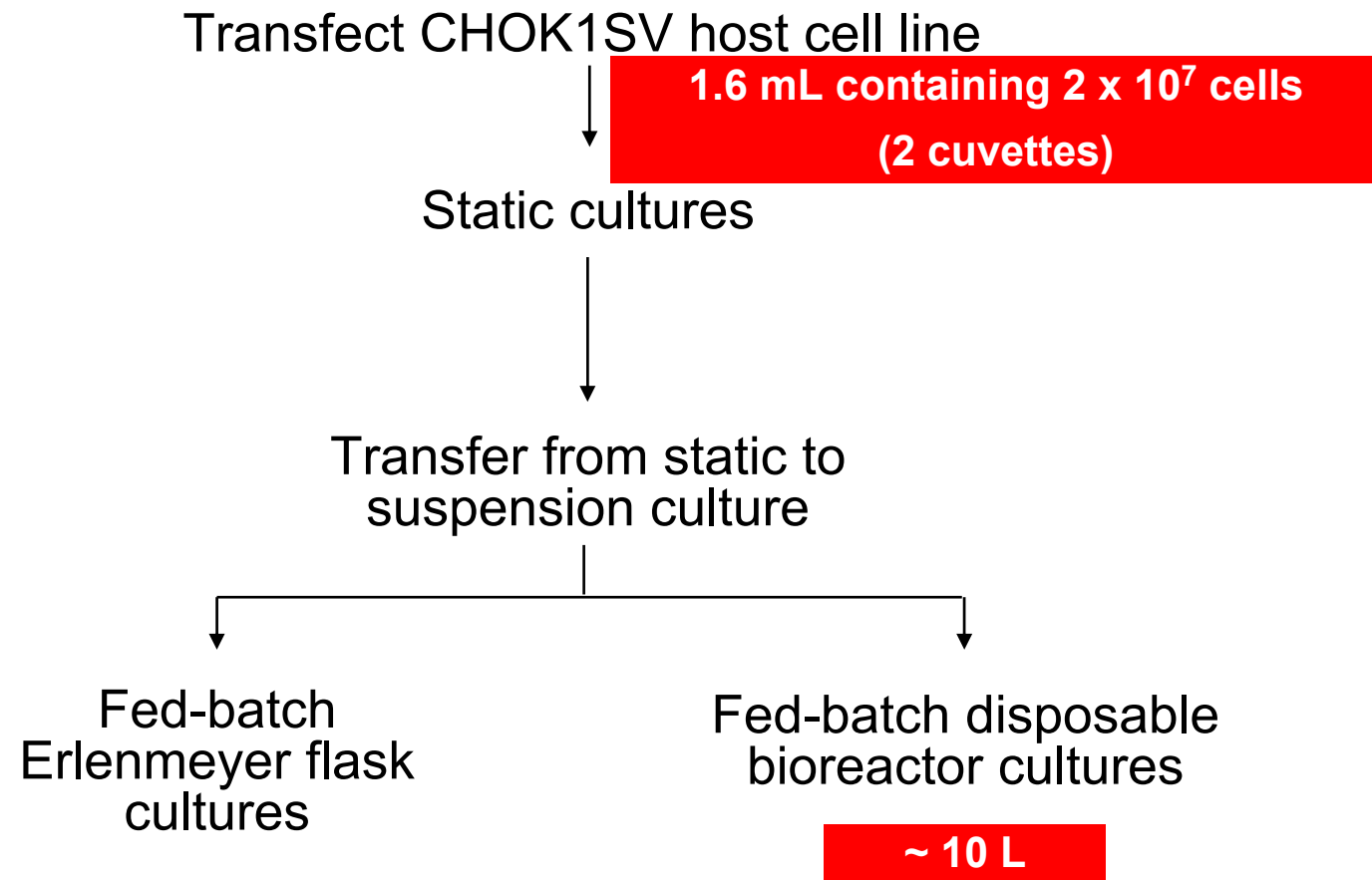
- Maintain as a pool of transfectants under selective pressure

Overview of Pooled Transfection



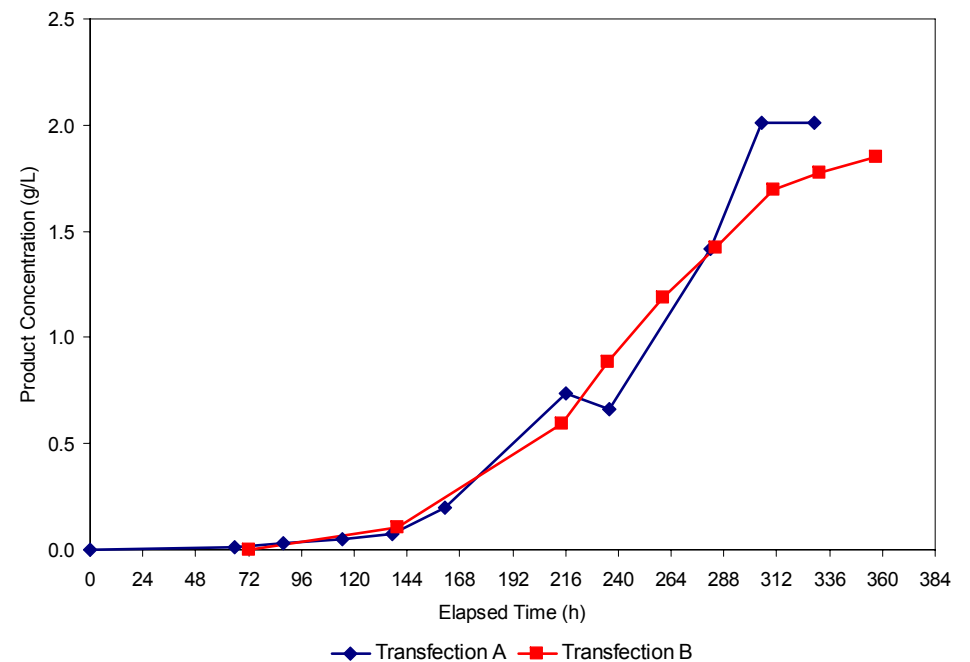
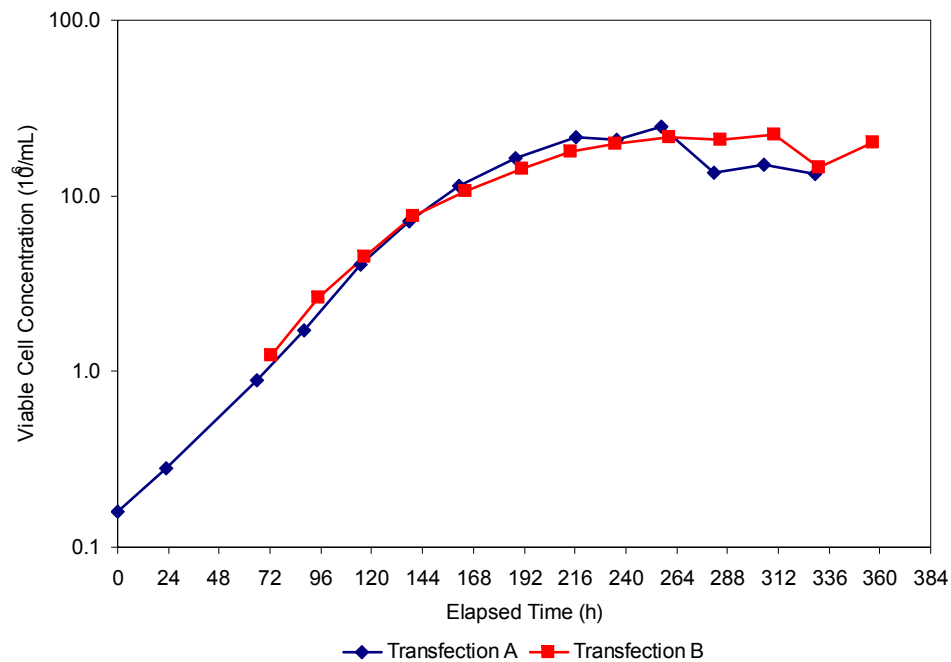
Transfection to harvest in 7 weeks

Culture Volumes



Transfection to harvest in 7 weeks

Growth and Productivity of GS-CHO Pools in Disposable Bioreactor



Inoculated 7 weeks after transfection (7 L culture)

Disposable Bioreactor Cultures of GS-CHO Pools

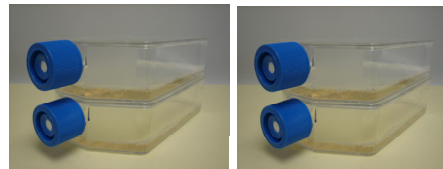
Run	Maximum viable cell concentration (10 ⁶ /mL)	Time integral of viable cell concentration (10 ⁶ cell.h/mL)	Specific growth rate (1/h)	Antibody concentration at harvest (mg/L)	Q _p (pg/(cell.h))	Culture length (days)	Day post transfection at start of culture
A	24.8	3443	0.027	2010	0.584	14	49
B	22.4	4103	0.023	1856	0.452	15	45

***~ 14 g of MAb harvested from 7 L cultures
9 weeks after transfection***

Reproducibility



Transfect CHOK1SV host cell line
100's of transfectants



4 x static cultures

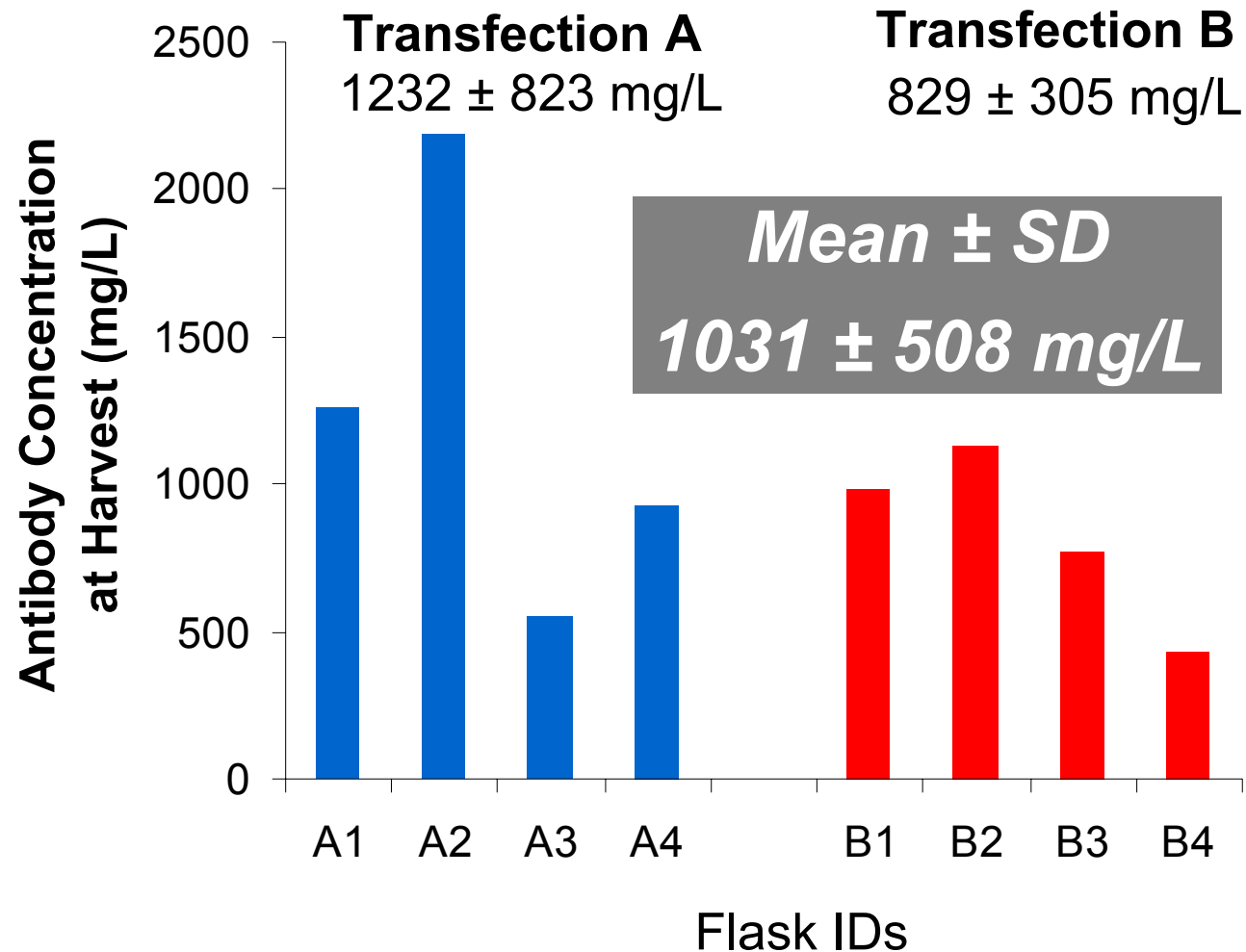


Transfer from static to
suspension culture



Establish duplicate fed-batch Erlenmeyer flask cultures

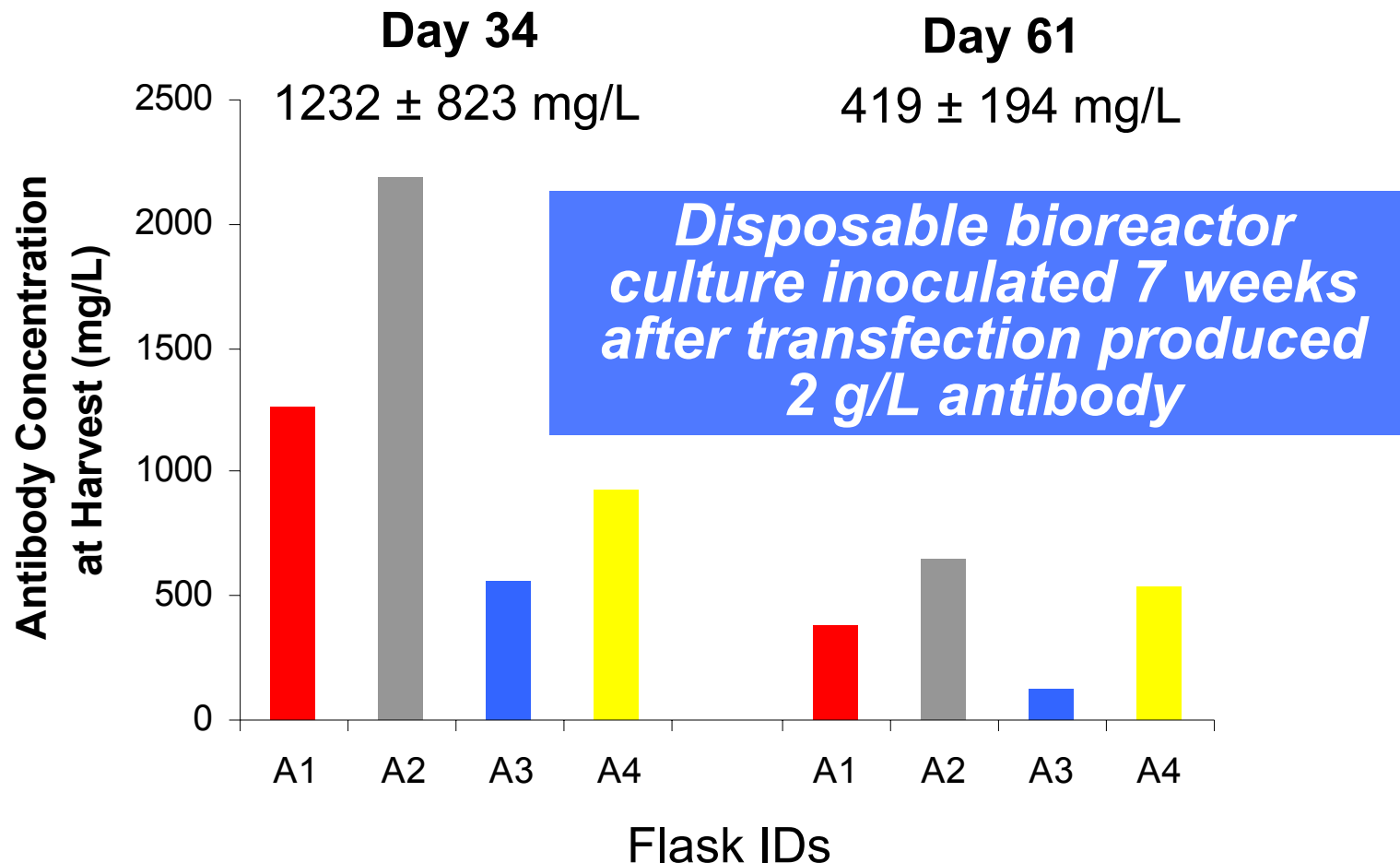
Is it Reproducible?



A Closer Look at Flasks Derived from Transfection A

Flask ID	Maximum viable cell concentration (10 ⁶ /mL)	Time integral of viable cell concentration (10 ⁶ cell.h/mL)	Specific growth rate (1/h)	Antibody concentration at harvest (mg/L)	Q _p (pg/(cell.h))	Culture length (days)
A1	12.2	2515	0.019	1262	0.502	15
A2	12.5	2755	0.020	2185	0.793	15
A3	14.3	2729	0.020	554	0.203	15
A4	11.9	2279	0.017	926	0.406	15

Duration of Model Antibody Expression by GS-CHO Pools



A Closer Look at the Early and Late Fed-batch Cultures of Transfection A

Changes Observed with Increased Time in Culture



Flask ID	Day post transfection at start of culture	Maximum viable cell concentration (10 ⁶ /mL)	Time integral of viable cell concentration (10 ⁶ cell.h/mL)	Specific growth rate (1/h)	Antibody concentration at harvest (mg/L)	Q _p (pg/(cell.h))	Culture length (days)
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A4	34	11.9	2279	0.017	926	0.406	15
A1	61	16.6	1911	0.022	377	0.197	11
A2	61	15.9	1942	0.023	642	0.331	11
A3	61	15.2	1788	0.024	126	0.070	11
A4	61	15.8	1820	0.022	532	0.292	11

Product Quality Assessment

- Samples analysed from
 - flasks derived from same transfection
 - flasks derived from different transfections
 - early and late fed-batch Erlenmeyer flask cultures
 - disposable bioreactor cultures
 - and compared to cB72.3 produced by a stable cell line, LB01, in a bioreactor

- Banding patterns were comparable by
 - reduced and non-reduced SDS PAGE
 - isoelectric focusing

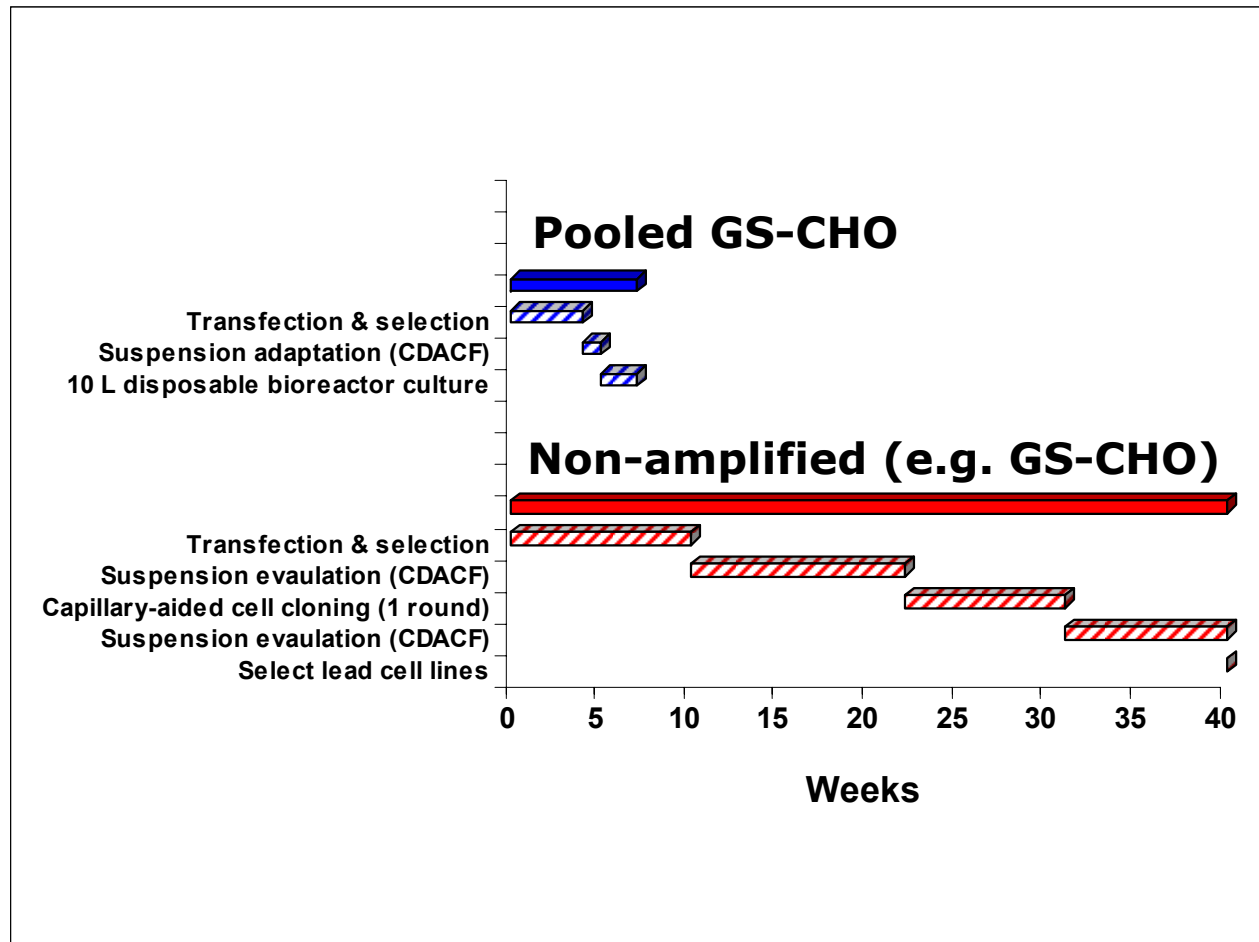
- Additional studies will examine product quality in more detail

Strategies for Scale Up

- Transfect more cells
 - 2 cuvettes → 10 L = 20 g MAb cB72.3 in 7 weeks
 - 10 cuvettes → 50 L = 100 g MAb cB72.3 in 7 weeks

- Additional sub-culture steps for GS-CHO pools in suspension
 - Increase volume available for inoculation of bioreactors

Approximate Timelines for Pooled GS-CHO Cells and Non-Amplified Clonal Cell Lines



Comparison with Transient Gene Expression

- Transient gene expression using polyethylenimine (PEI) or calcium phosphate useful for the production of small amounts of protein (up to 10's mg/L) within weeks
 - Amount of DNA required?

- At least 100 x less DNA required for GS-CHO pools¹

- GS-CHO pools achieve higher cell specific productivities due to stringency of initial selection

- Assuming 50% recovery of purified MAb, a 5 L culture would be required to produce 5 g of the model antibody cB72.3 using GS-CHO pools compared with a 250 L culture using transient gene expression (assuming productivity of 40 mg/L).

¹Galbraith, D. J., Tait, A. S., Racher, A. J., Birch, J. R., and James, D. C. (2006) Control of Culture Environment for Improved Polyethylenimine-Mediated Transient Production of Recombinant Monoclonal Antibodies by CHO Cells. *Biotechnol. Prog.*

Summary

- 14 g of MAb cB72.3 harvested from disposable bioreactor cultures operated in fed-batch mode established up to 7 weeks after transfection
- Preliminary product quality assessment showed that cB72.3 antibody produced by GS-CHO pools in either Erlenmeyer flask cultures or a disposable bioreactor and cB72.3 antibody produced by stable GS-CHO cell line in a bioreactor were comparable
 - Additional studies will be performed
- Further scale up possible
 - Potential for 10's to 100's of g of material
- Potential to accelerate clinical candidate optimization

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