



Lonza

DEVELOPING HIGHLY PRODUCTIVE CELL LINES AND CELL CULTURE PROCESSES

Dr Robert Gay

© Lonza Biologics plc, 2004

- Biopharmaceuticals represent 10-30% of all new drugs in recent years
 - rMAbs have grown from 1% of the biopharmaceutical market in 1995 to 14% in 2001
- Polastro and Tulcinsky, SCRIP Magazine Sep 2002
- Currently fifteen rMAbs on the market with many more in development
 - Several are 'blockbuster' therapeutics
 - High dose requirement – 10s to 100s Kg per annum required
 - Challenge: produce large quantities with cost and time efficiency

- Can large quantities simply be obtained by scaling up and up?


- Cell lines
 - Highly productive
 - Stable

- Cell culture processes
 - Robust
 - Scalable

20,000 Litre Large Scale Reactor

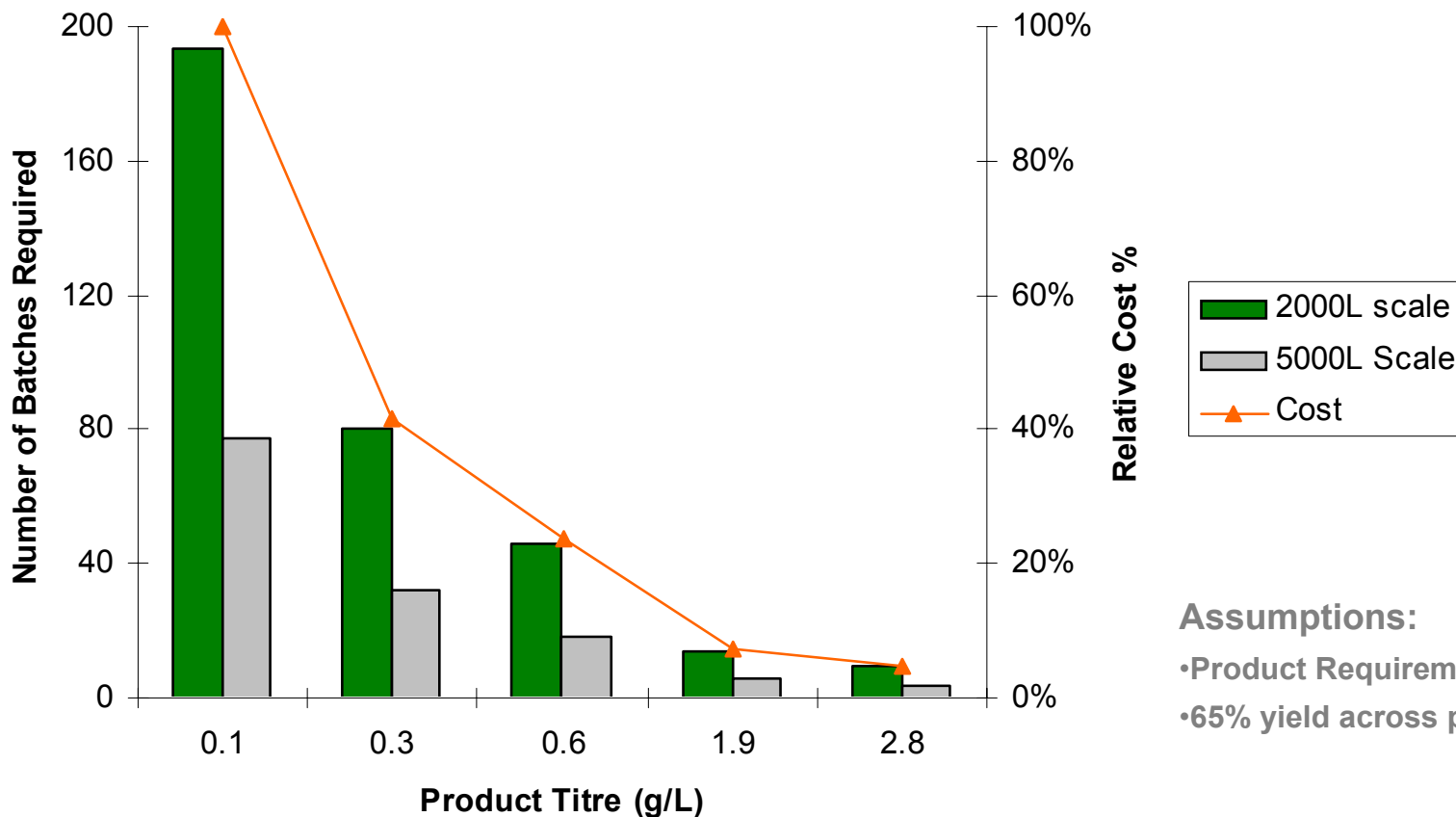
Lonzo



- 
- Can large quantities simply be obtained by scaling up and up?
 - Cell lines
 - Highly productive
 - Stable
 - Cell culture processes
 - Robust
 - Scalable


The Benefits of Increasing Productivity

Lonzo



Assumptions:

- Product Requirement: 35kg
- 65% yield across purification

- 
- Host cell
 - Expression system
 - Transfection and selection protocol
 - Rapid creation

 - Goal to create stable, high producing cell lines
 - Grow in suspension culture
 - Grow in a chemically defined, animal component-free media
 - Optimise culture
 - High specific productivity, high cell numbers which can be maintained for extended time

- Host cell CHOK1SV
- Expression system GS
- Electroporation and selection protocol MSX
- Rapid creation 20 weeks

- Early phase clinical supply (Uncloned)
 - cDNA to cGMP in a generic process in <12 months

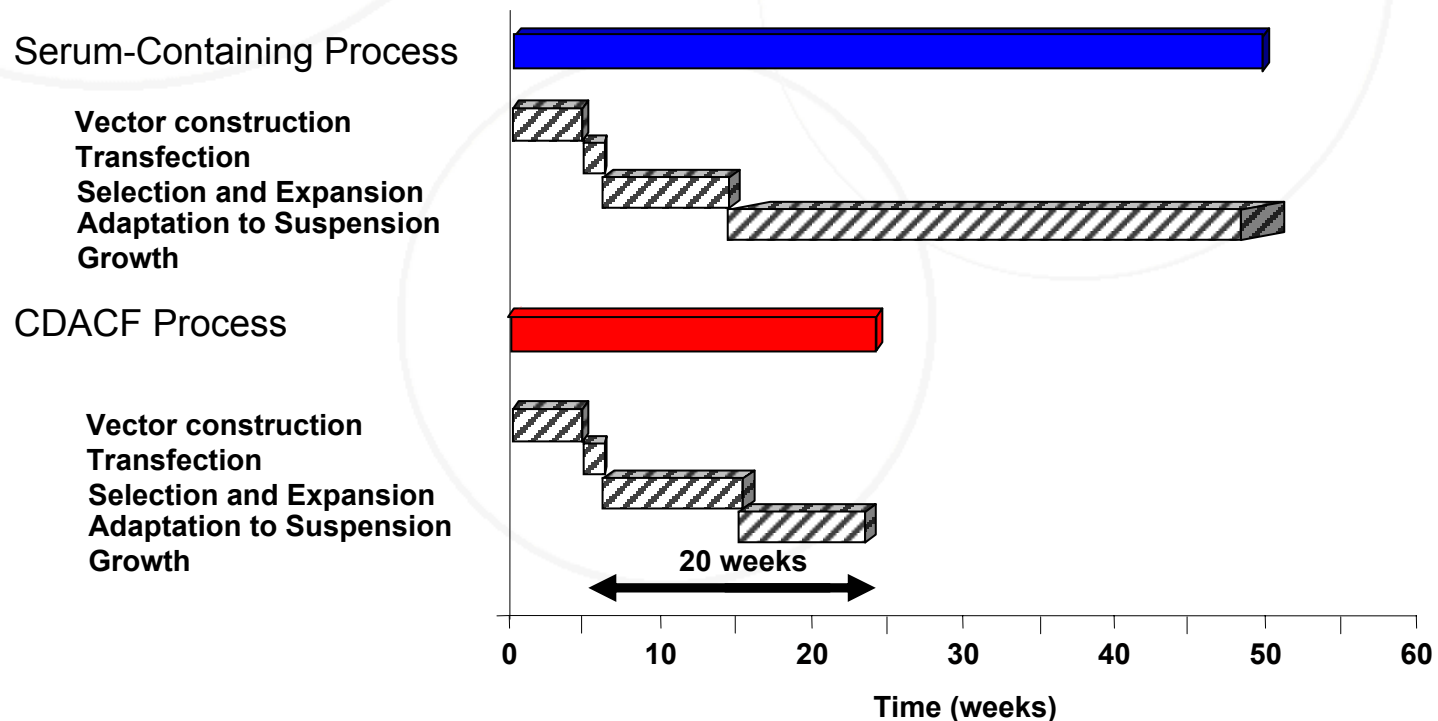
- Late phase clinical supply (Clonal)
 - Marry cell line with optimised process

- Developed CHOK1SV (suspension variant)
 - Grows as single cell suspension
 - Pre-adapted to growth in chemically defined, animal component-free media
 - Exhibits good growth characteristics
 - Reach high maximum viable cell concentration
 - Able to maintain cultures at high cell viability

- The GS Gene Expression System

Timeline Reduction with CHOK1SV

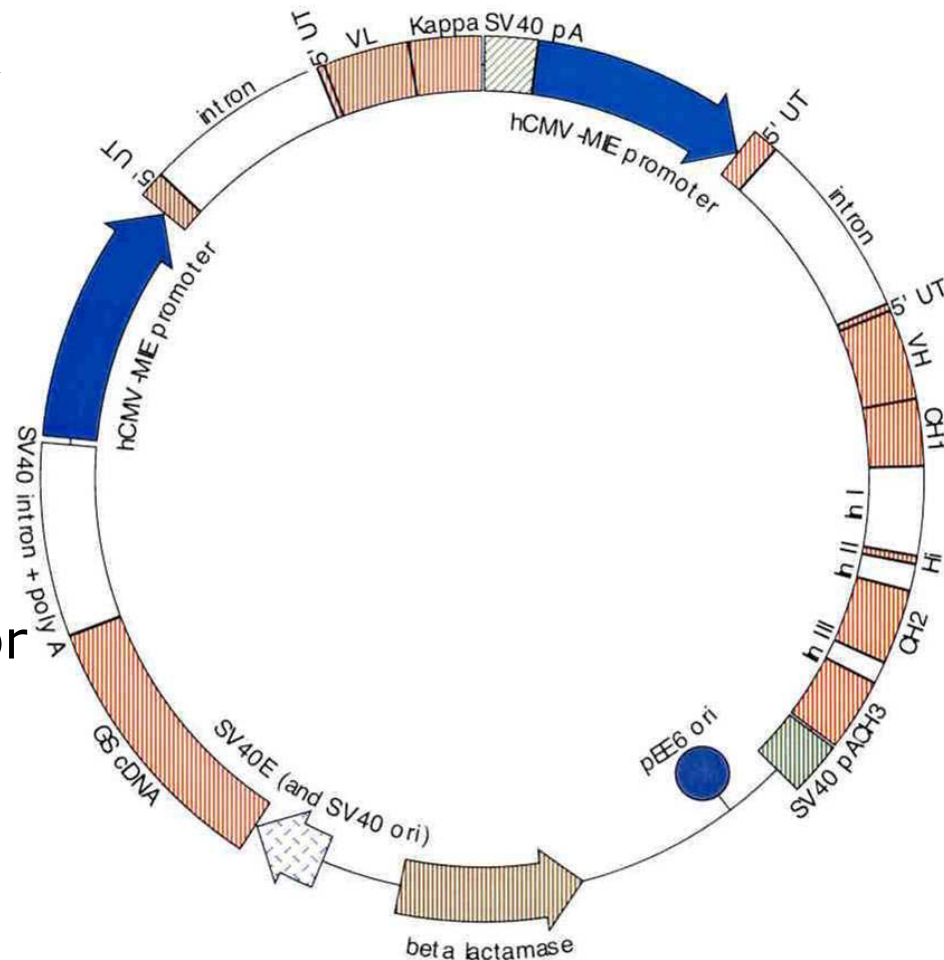
- Use of suspension variant of CHO K1 pre-adapted to growth in chemically defined, animal component-free (CDACF) media substantially reduces time taken to generate cell lines



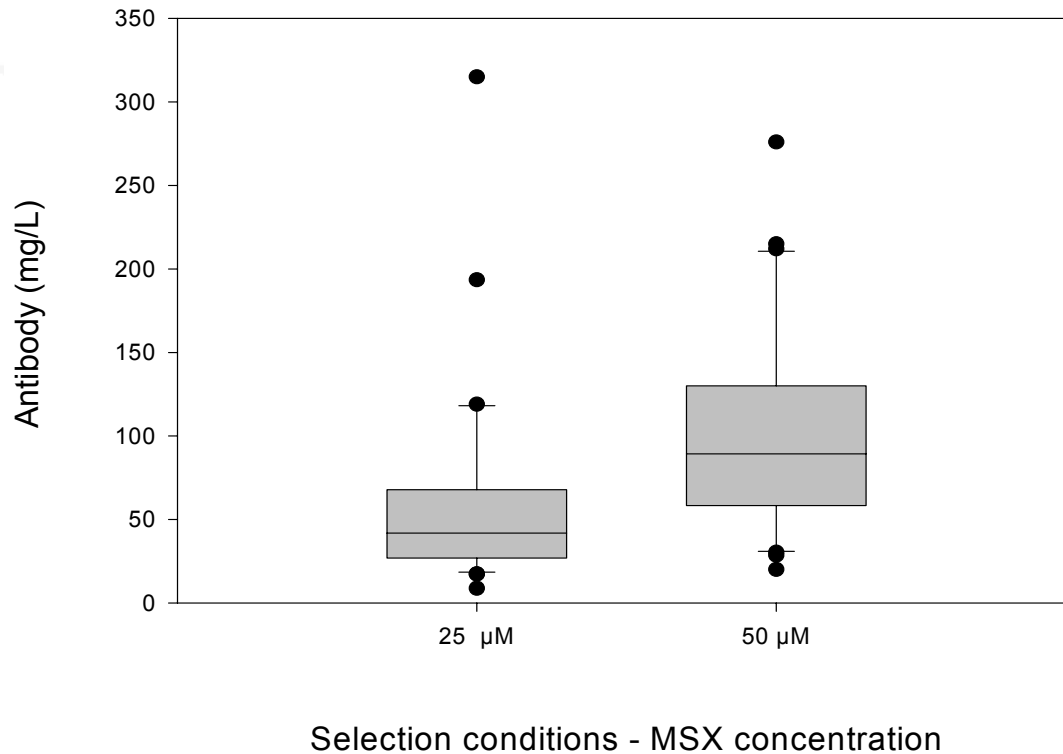
- Developed CHOK1SV (suspension variant)
- The GS Gene Expression System
 - Glutamine synthetase (GS) used as a selectable marker
 - Glutamine omitted from culture media as selective pressure
 - Further selection pressure applied with methionine sulphoxamine (MSX) - a specific inhibitor of GS
- Focus on recent developments creating GS-CHO cell lines

GS Expression Vectors

- Antibody genes driven by strong promoters
- GS gene driven by weak promoter
- Biases for selection of rare integration into transcriptionally active sites in genome
- Both light chain and heavy chain on one vector
- Range of constant region vectors available preformatted to facilitate antibody cloning



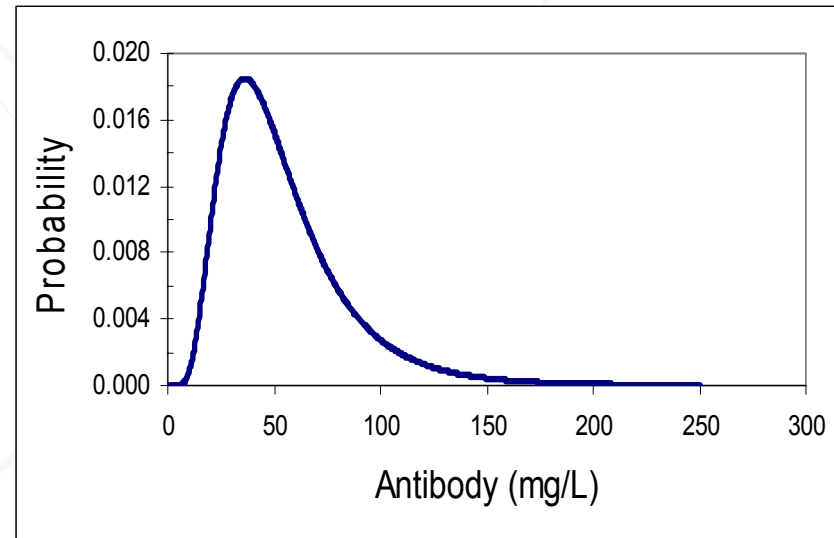
- Influence of selection conditions for GS-CHO cell lines with cB72.3 antibody



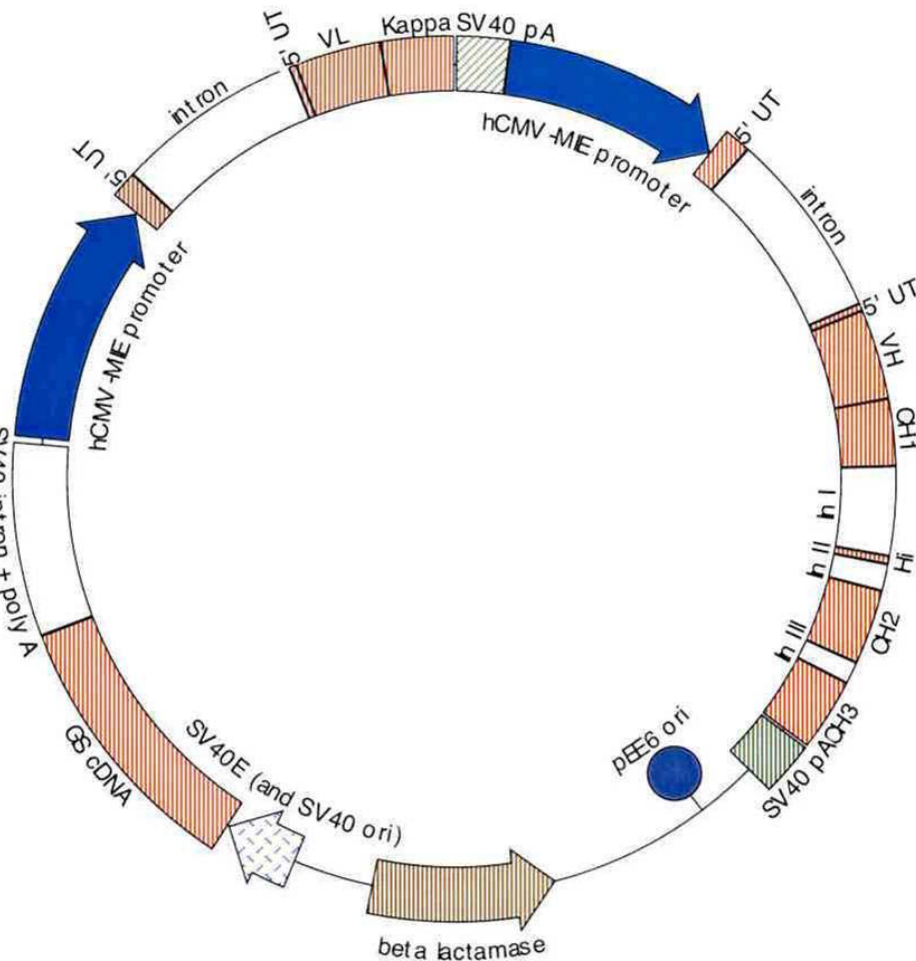
Cell lines have not been amplified.

High producers are infrequent

- Probability distribution of antibody productivities for primary GS-CHO transfectants (24 well plates)
- 90% transfectants produce less than 90 mg/L
- 1.5% transfectants produce more than 150 mg/L



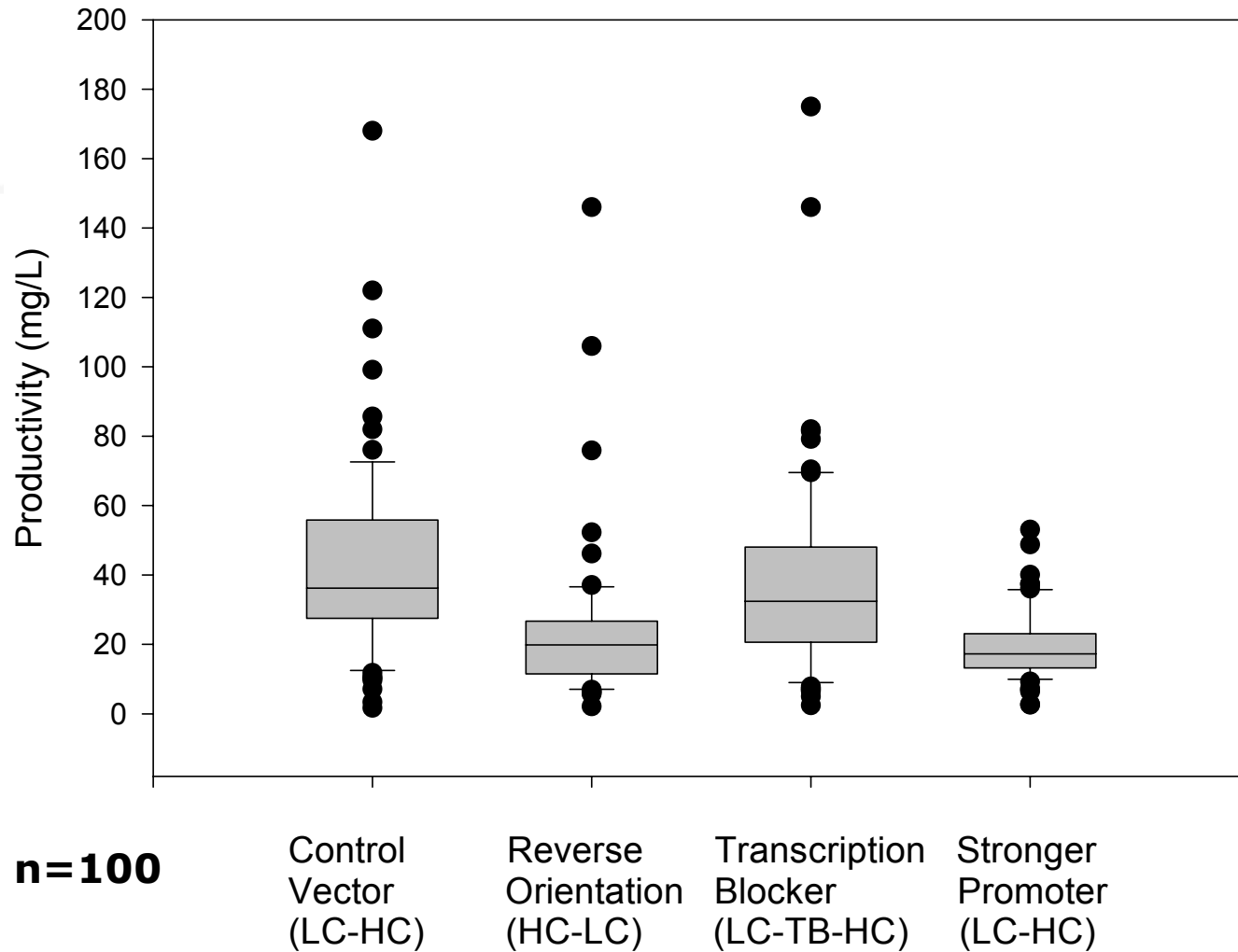
Improving GS Vectors for Antibodies



- Free light chain often seen in cultures
- Does first gene (LC) interfere with expression of second gene (HC)?
- Can levels of LC and HC be balanced?
 - Transcription blocker
- Must the LC gene be first?
 - Reverse the order and put HC first
- Can stronger promoters be used?

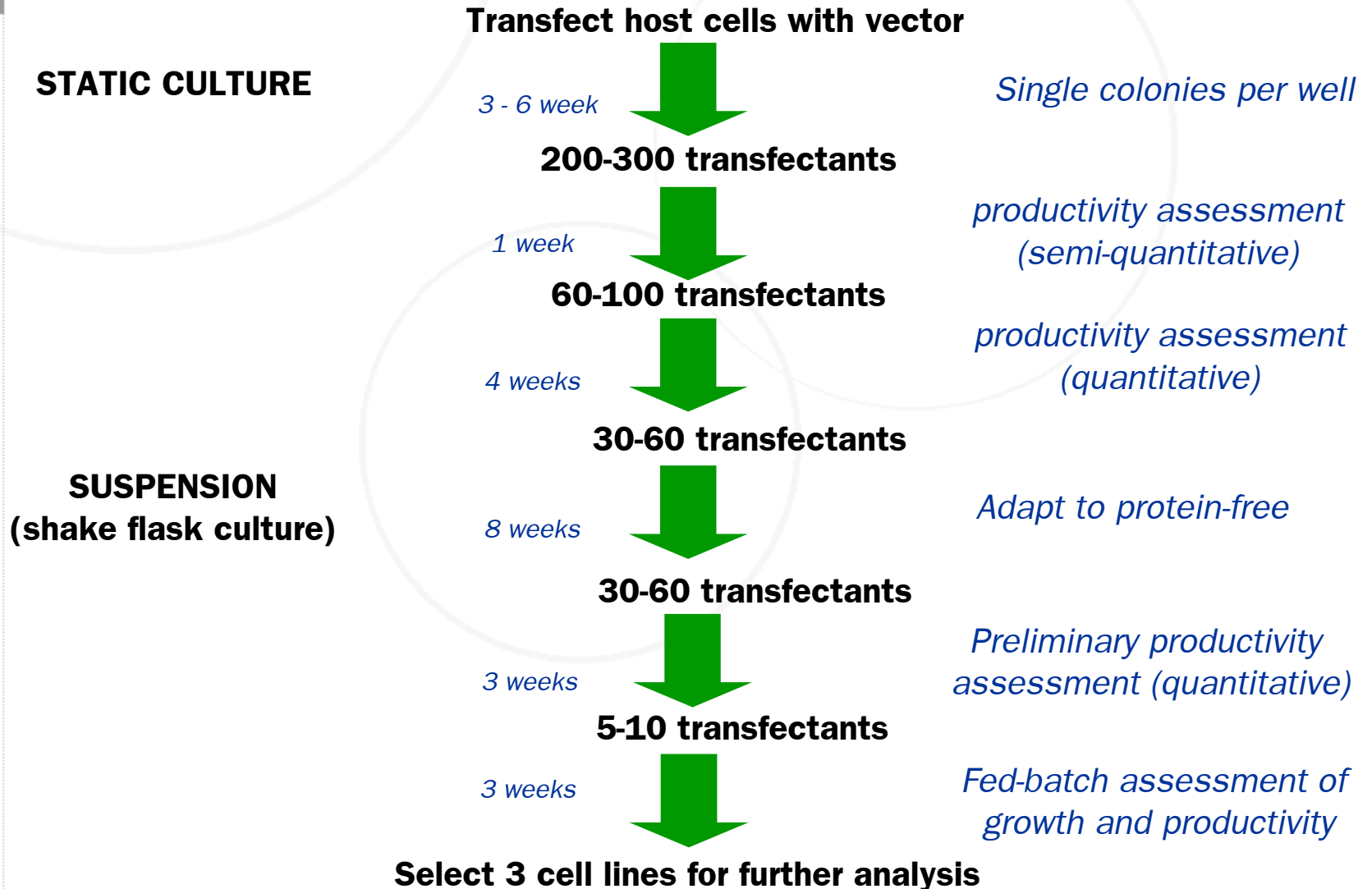
Improving GS Vectors for Antibodies

LONZA

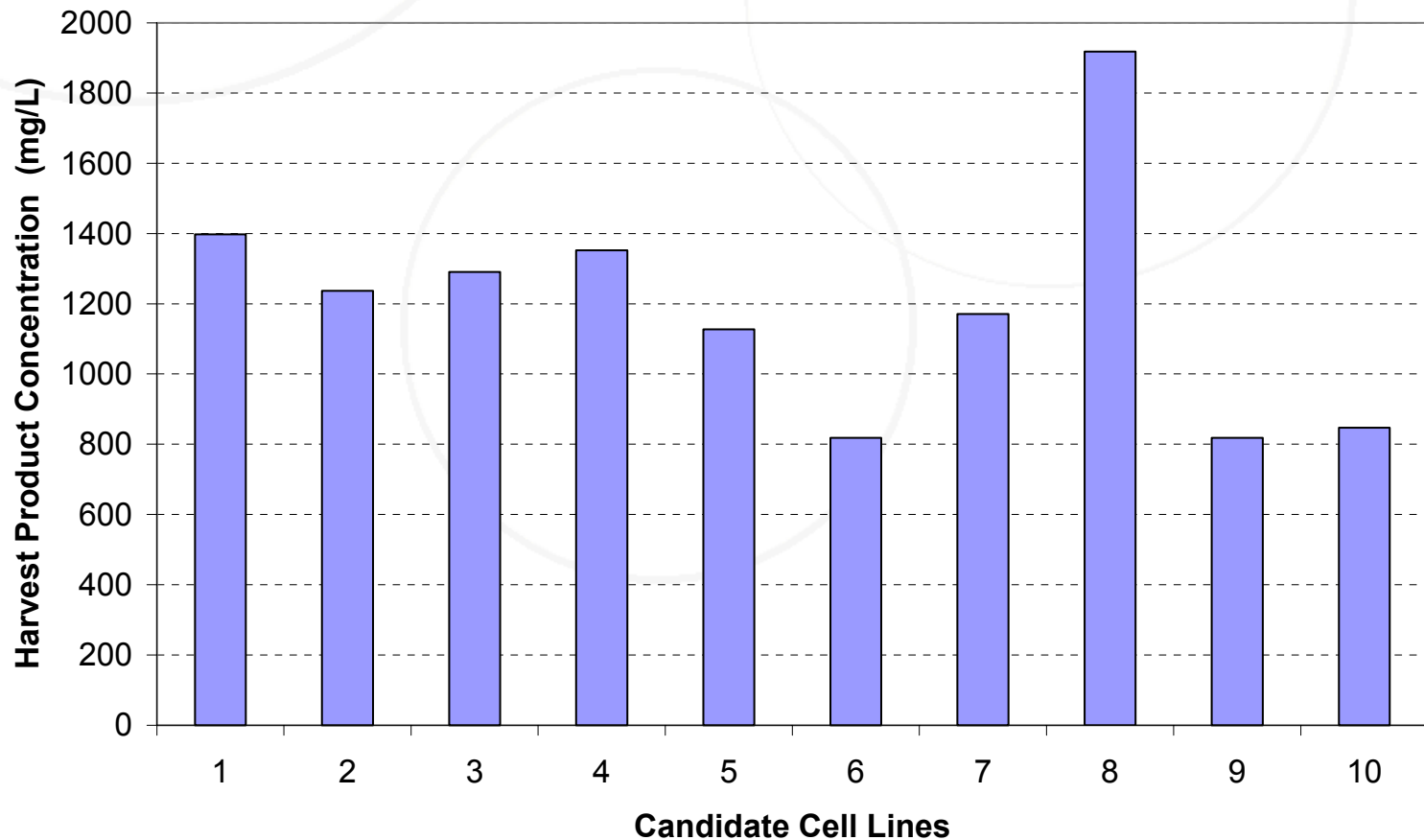


Finding High Producers I

LONZA

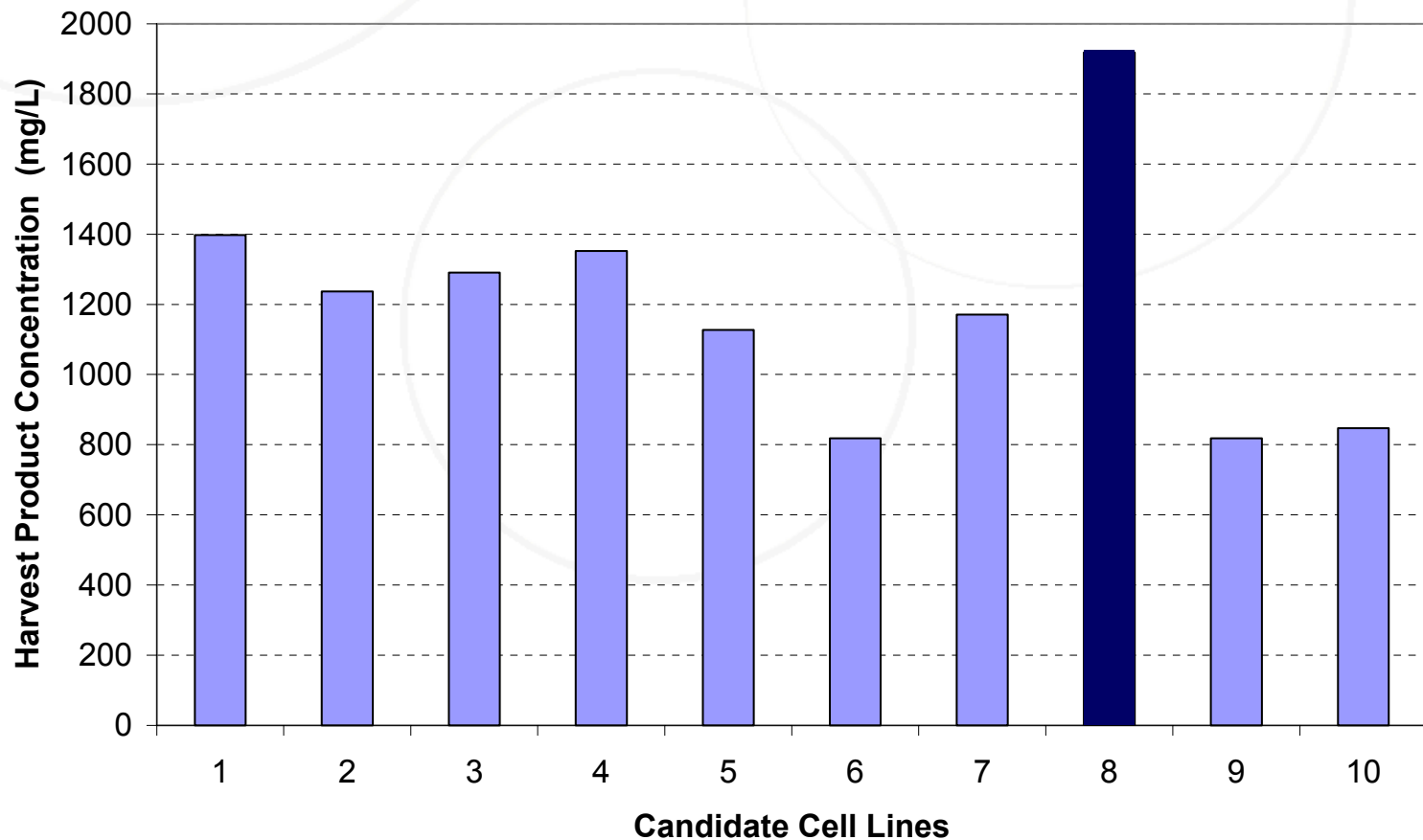


■ Shake-flask cultures operated in fed-batch mode



Finding High Producers II

■ Shake-flask cultures operated in fed-batch mode



Finding High Producers III

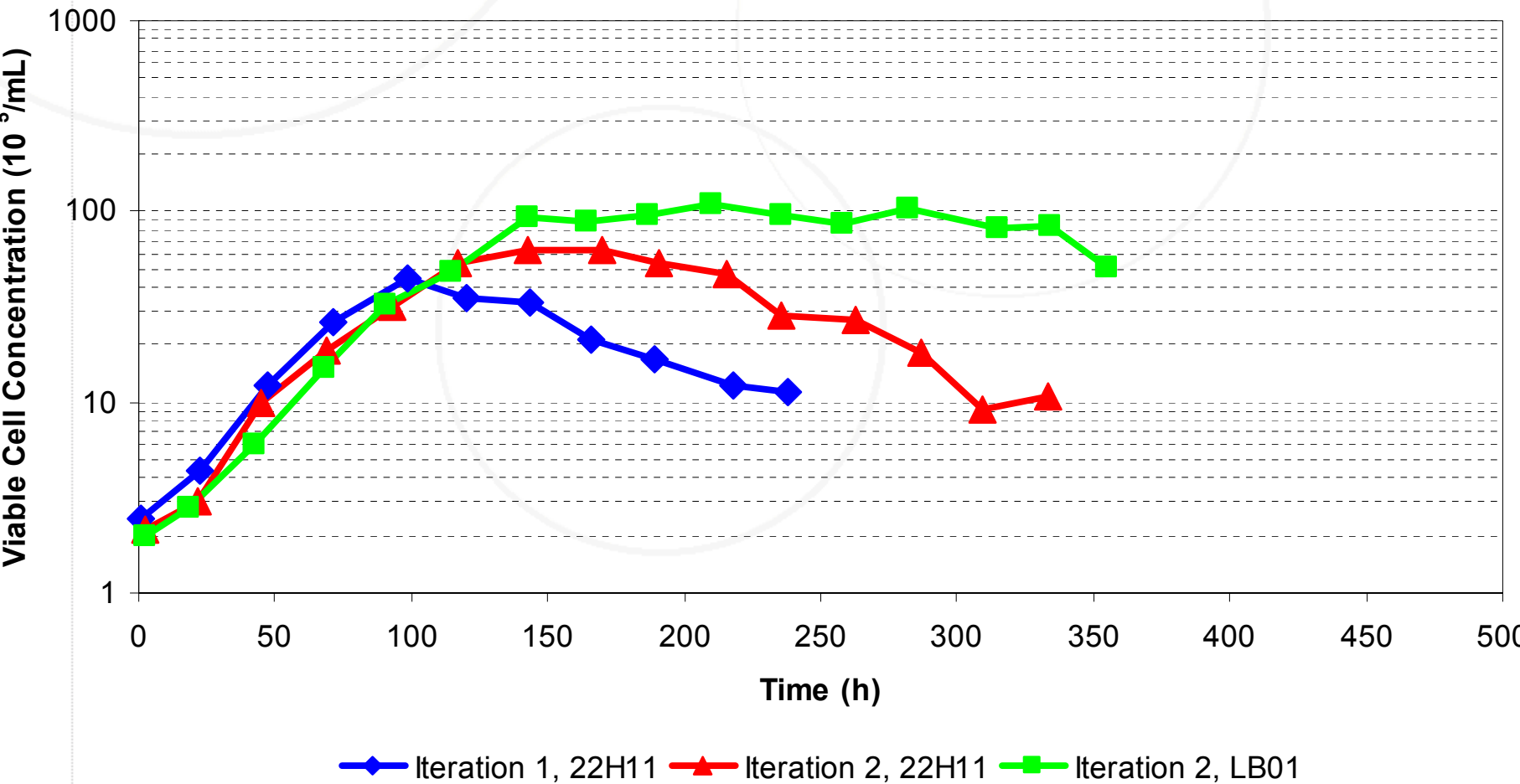
LONZA

Bioreactor	Maximum Viable Cell Concentration (10⁶ cells/mL)	Product Concentration (g/L)	Specific Production Rate (pg/cell/h)	Harvest day
Laboratory-scale (10 L)	9.42	1.6	0.78	15
Pilot-scale (130 L)	10.78	1.9	0.76	17
Manufacturing (200 L)	9.66	1.4	0.78	15

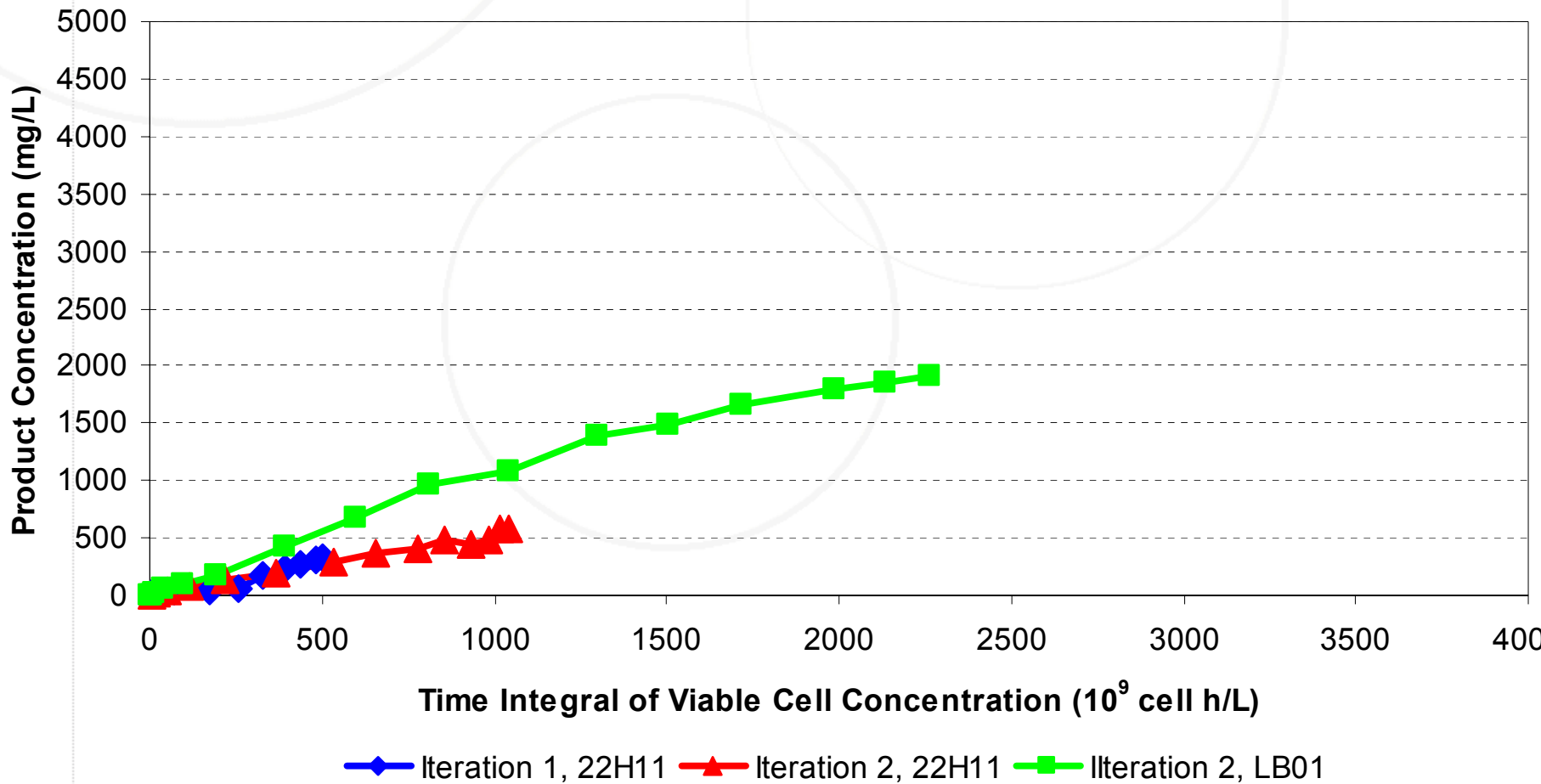


- Significant potential to improve processes
 - Medium design and feeding strategies
 - Physicochemical environment
 - Iterative process
- Full chemical definition
 - Knowledge of media components gives full control of process optimisation
 - No lot to lot variability of raw materials
 - Simplification of downstream processing
- Animal component-free
 - Potential source of adventitious agents and product contaminants

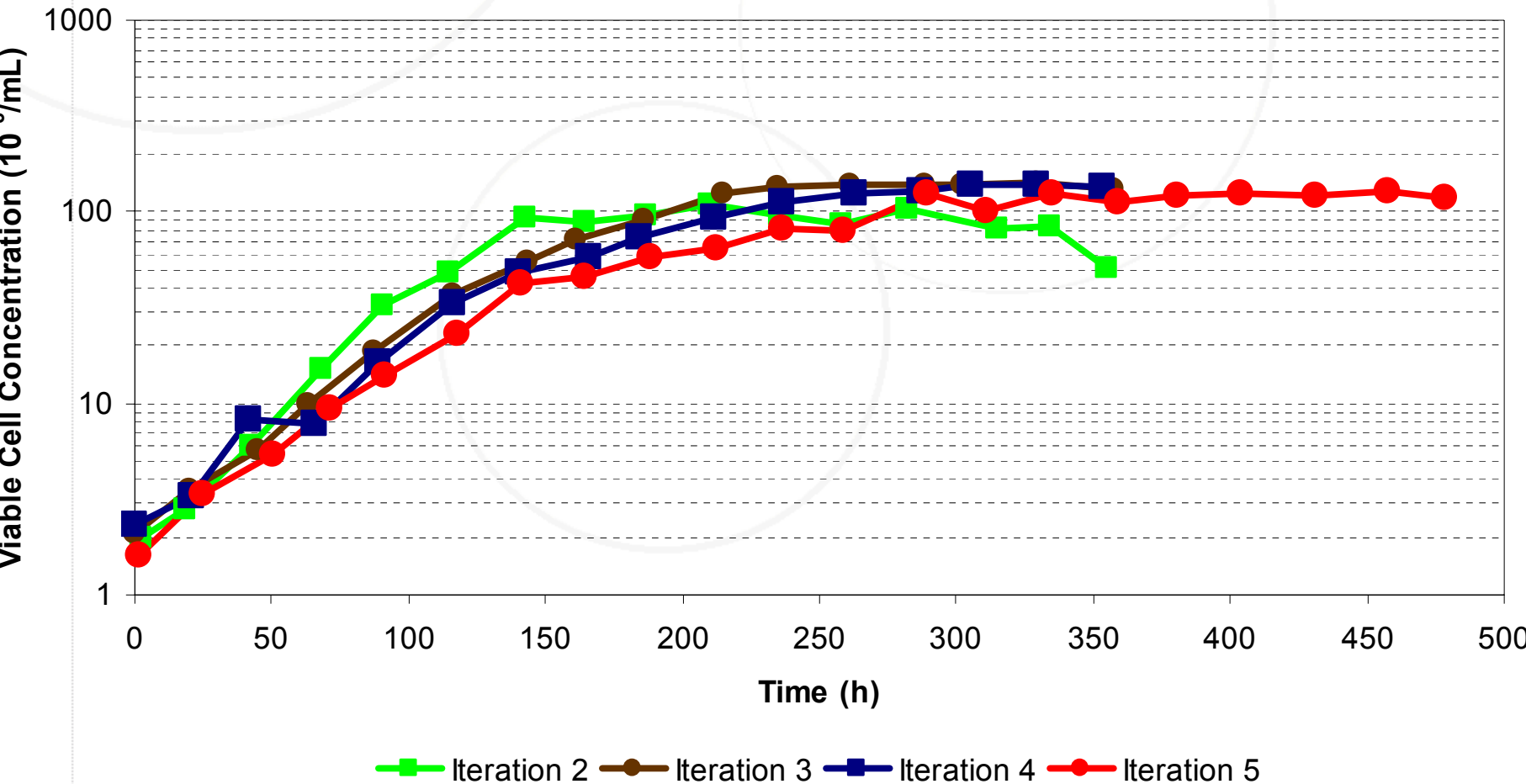
Improving Cell Growth



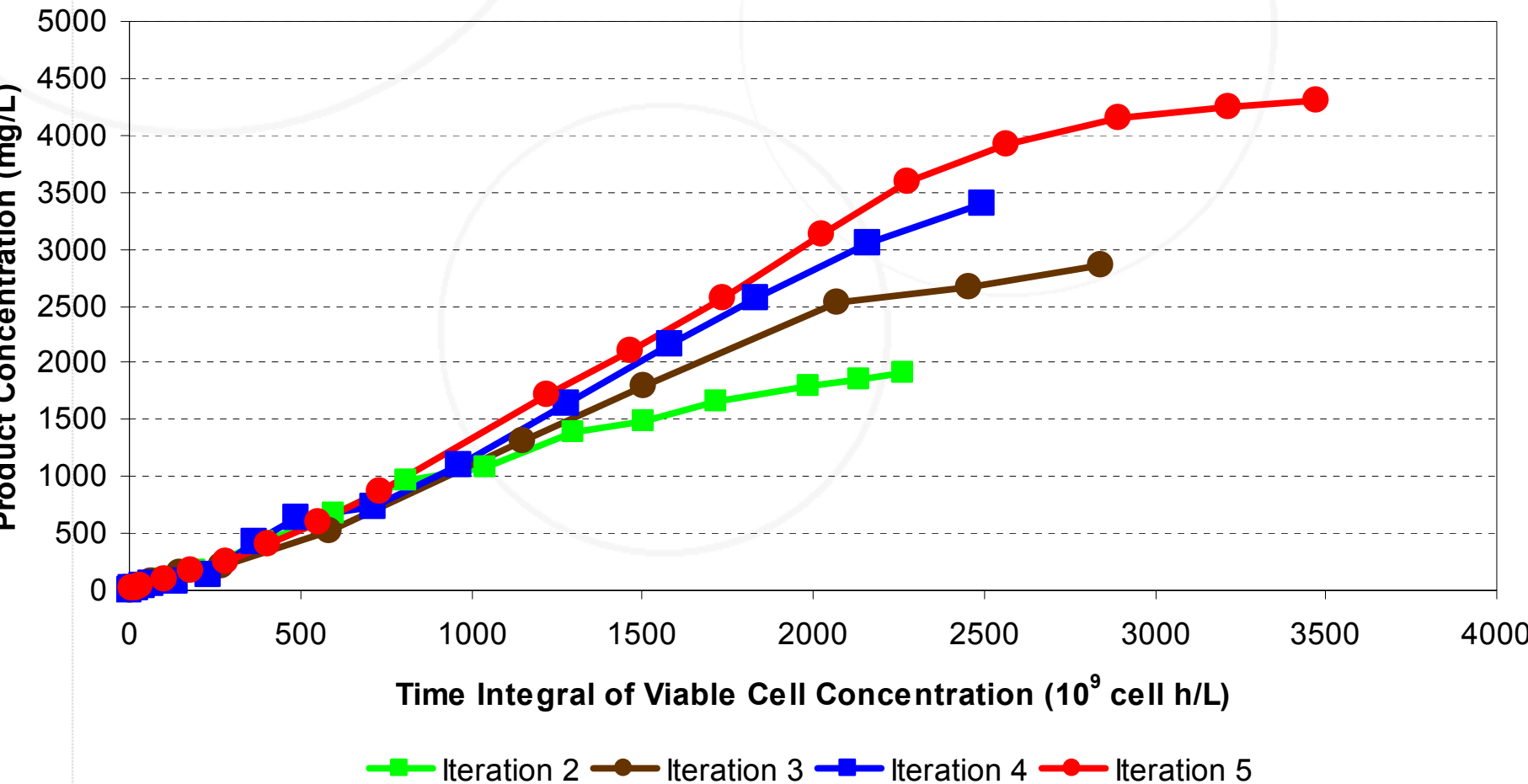
Improving Product Accumulation



Improving Cell Growth



Improving Product Accumulation



CHO Process Optimisation Summary

LONZA

Process	Antibody (mg/L)	Fold increase
Original cell line	139	
Iteration 1	334	2
Iteration 2	585	4
New cell line	1917	14
Iteration 3	2829	20
Iteration 4	3560	26
Iteration 5	4301	31

- Creation of highly productive cell lines is the sum of many parts
 - Host cell and expression system
 - Transfection and selection
 - Strategies to identify the highest expressers

- Significant potential for further yield enhancement
 - Process optimisation
 - Lonza's philosophy of generic platform development based on chemical definition and animal component-free processes

Acknowledgements

Lonza

- Development – Lonza Biologics Slough, UK
 - Molecular Biology
 - Cell Culture Process Development
 - Assay Development
 - Process Scale Up and Support
- Rinat Neuroscience Corp.

DEVELOPING HIGHLY PRODUCTIVE CELL LINES AND CELL CULTURE PROCESSES

Dr Robert Gay – Lonza Biologics
224 Bath Road, Slough, UK

Email – robert.gay@lonza.com

© Lonza Biologics plc, 2004