

**A Case Study:
Multiple process
improvements
implemented during
clinical development ...**



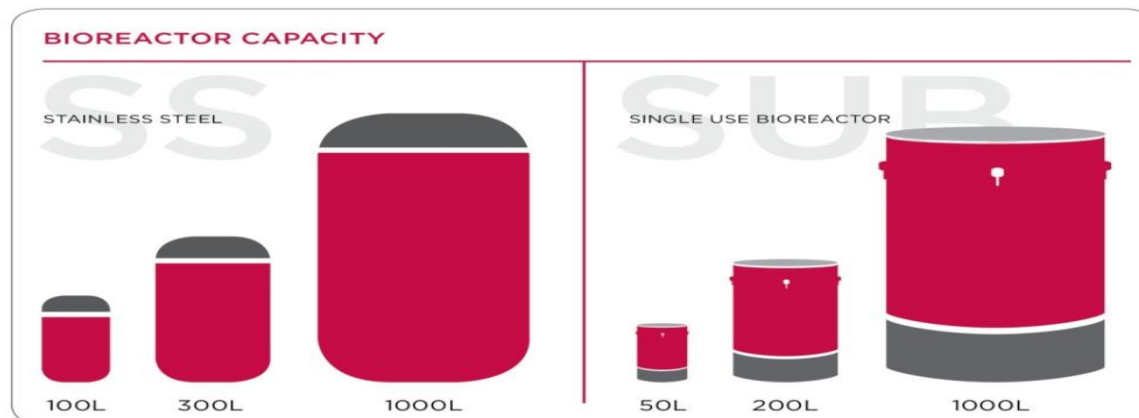
**... resulting in 10X
production yield with
consistent product
characteristics**

**BioProcess International Conference
Boston, 2013**

**Connie Chang, Director of Process Sciences
Michael Brown, Associate Scientist, Process Sciences**

Avid Bioservices Company Overview

- CMO business established in 2002
- Significant experience in mammalian cGMP production
 - Hundreds of cGMP lots produced to date
 - Commercial production since 2005
 - >18 successful FDA and European inspections including multiple PAIs
- Innovative approach to manufacturing
 - Stainless Steel Bioreactor (SSB) production since 1997
 - Single Use Bioreactor (SUB) production since 2008
 - ❖ 1st CMO on west coast to implement SUB
 - Implementing disposable components in upstream and downstream processes



Today's Case Study

Outline

- Multiple process changes during clinical development
 - Scale-up
 - Change of cell line + downstream optimization
 - Implement SUB for manufacturing process
 - Optimize media and feed

Continuous Process Improvement to Support Clinical Development

Phase I

- Adequate but low titer process
- Support small clinical studies

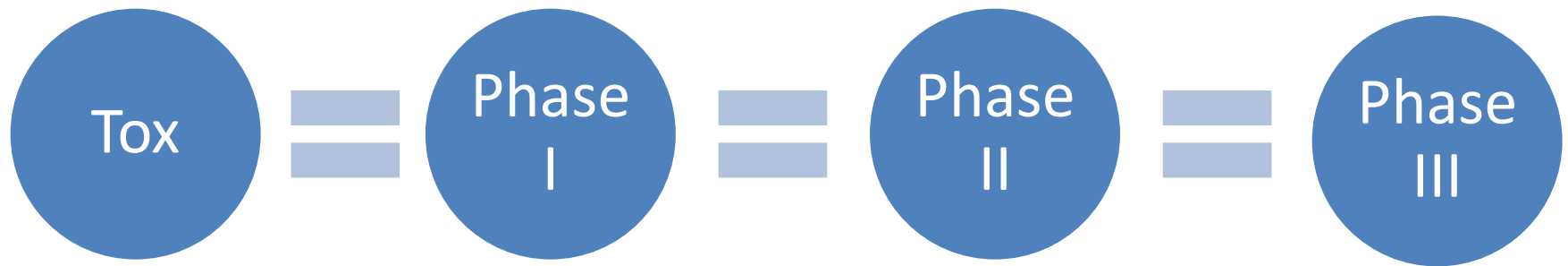
Phase II

- Higher Yields Needed
- Support mid sized clinical studies

Phase III

- High Yield Optimized Process
- Support Phase III and commercialization

Regulatory Strategy: Maintain High Degree of Product Similarity When Implementing Any Process Changes



- Maintain Continuity of Clinical Data
 - Similar product throughout clinical development strengthens the package
- Eliminate need for human bridging studies or need to repeat toxicology studies
- Our approach is based on strong characterization package
 - Analytical Characterization
 - Small animal PK
- Our philosophy is NO manufacturing changes during Phase III and minimal modifications to support commercialization

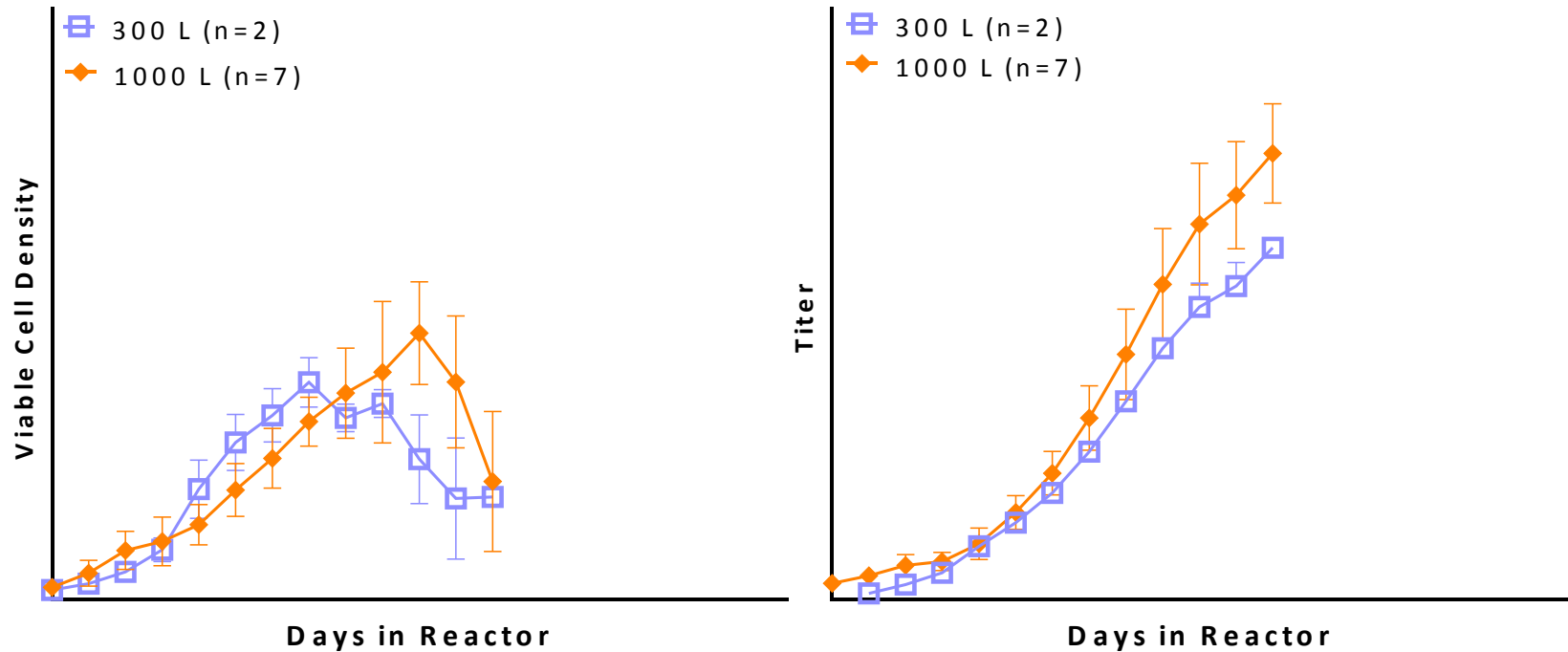
Phase I / Early Phase II Clinical Production

Process Scale-Up During Phase I and Early Phase II

- 1st generation process
 - 300 L Stainless Steel Bioreactor with a fed batch process
 - Animal component free media
 - One 300 L Stainless Steel Bioreactor yielded 150 clinical doses
- Process Scale-up
 - 300 L Stainless Steel Bioreactor to 1,000 L Stainless Steel Bioreactor
 - Supported Phase II clinical studies



Comparison of 300 L vs. 1,000 L Production



- Achieved similar growth and titer between two different scale productions

Degree of Similarity Analysis: 300 L to 1,000 L Scale-up

- Phase-appropriate analytical testing assessed comparability between the 300 L and 1,000 L drug substance

Lot Release and Characterization Assays	
Antigen Binding	Oligosaccharide Analysis
Monomer Content	Peptide Map
IEF	N-terminal Sequencing
SDS-PAGE	Stability

- Reports submitted to the FDA demonstrated a high degree of similarity between 300 L and 1,000 L Stainless Steel Bioreactor scales
- Process scale up resulted in the 1st generation process at 1,000 L scale producing **3X more** clinical material to support early Phase II

Phase II Clinical Production

Increasing Clinical Needs Necessitated Process Changes

- Phase II clinical trials require 1,000+ doses
- Cost of goods becoming another key consideration
- Later stage clinical studies may require thousands of doses

A number of process changes were implemented to address these needs and to improve manufacturing flexibility

- New cell line with greater potential for producing higher titer
- Downstream process improvements to improve purity profile
- Implement the use of 1,000 L SUB to give production flexibility through ability to produce in either Stainless Steel Bioreactors or SUB

2nd Generation Changes Designed to Increase Yield

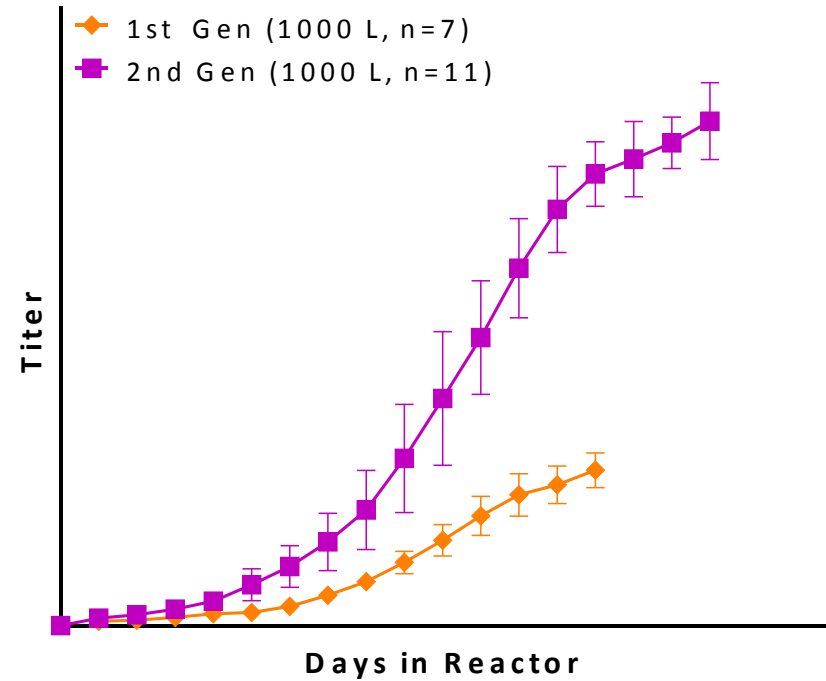
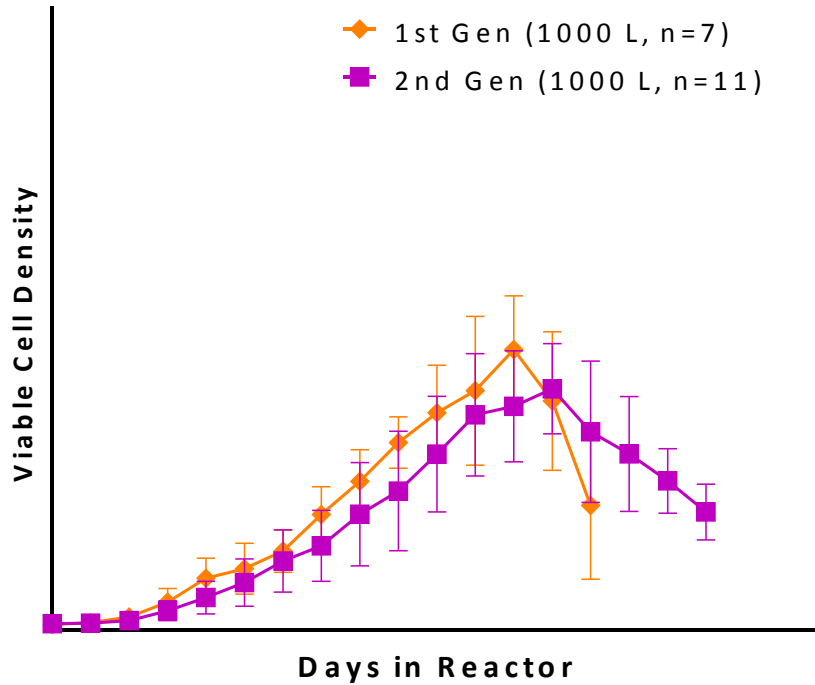
Cell Line and Process Changes:

- New transfection and subcloning created a new cell line (2nd generation cell line)
 - ~3X more product than the 1st generation process
 - Animal component free fed batch
 - Produced in 1,000 L Stainless Steel Bioreactor
- In addition, modernized downstream process to increase product purity

Implementing SUB into manufacturing process:

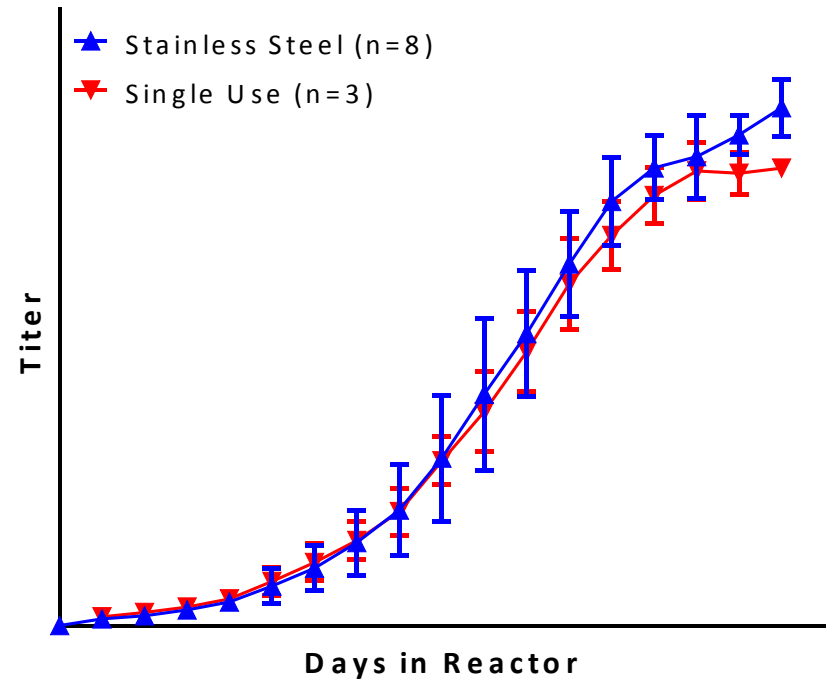
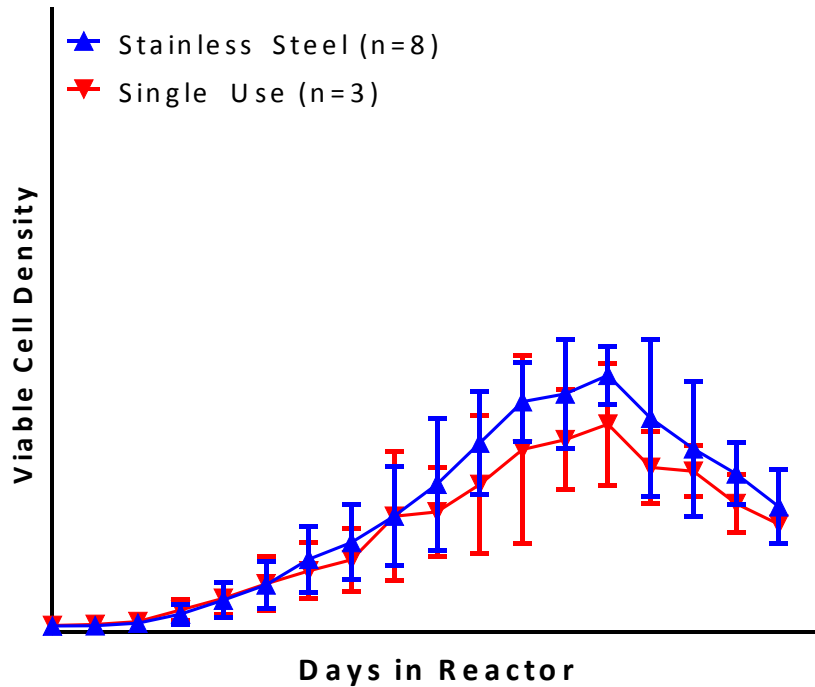
- Established production in 1,000 L SUB

Comparison of 1st and 2nd Generation Cell Lines



- Similar cell growth
- 2nd generation cell line produced **~3X** higher titer

Comparison of 1,000 L Stainless Steel Bioreactor and 1,000 L SUB



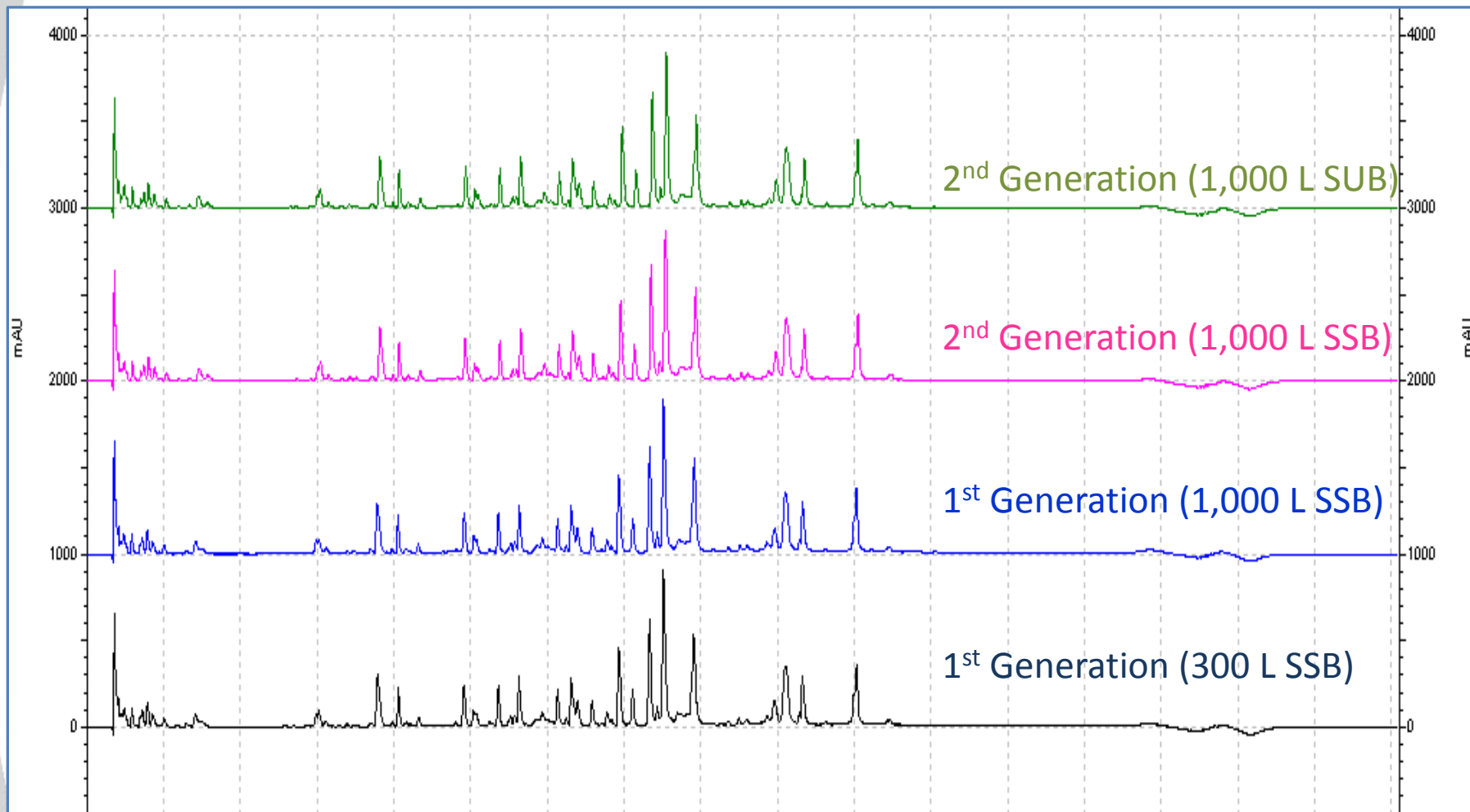
- Comparable upstream process
- Similar cell growth and titer between Stainless Steel Bioreactor and SUB

Product Comparability Analysis to Support 2nd Generation Production Process Changes

- Degree of similarity was demonstrated with lot release, analytical characterization and stability data

Lot Release for Drug Substance/Drug Product		Additional Characterization
General	Identity	Analytical Ultracentrifugation
Visual inspection	IEF/cIEF	N-terminal Sequencing
Color and Appearance	Peptide Map	C-terminal Sequencing
Particulate Matter (DP)		Monosaccharide Composition
pH		Neutral Sugar Assay
Strength and Potency	Process-Related Impurities (DS)	Stability Studies
Protein Content	Residual Protein A	Non-Clinical Pharmacokinetics in Small Animal Model
Antigen Binding	Residual DNA	
Potency Bioassay	HCPs	
Safety	Purity	
Endotoxin	SEC	
Bioburden	IEX	
	SDS-PAGE	
	Oligosaccharide Analysis (DS)	

Example of Comparable Product Characteristics Assessed by Peptide Map



Tox



Phase
I



Phase
II

Summary of Changes Made During Phase II

- Process changes made during Phase II resulted in a product which met BOTH analytical and animal PK bioequivalence criteria.
- Package submitted to FDA resulting in approval for:
 - Process changes
 - Cell line
 - Optimized downstream process
 - Interchangeable production in Stainless Steel Bioreactors and SUB

Phase III Clinical Production

Process Changes Made in Preparation for Phase III 2nd Generation Cell Line w/ Optimized Media and Feed Strategy

One single Phase III clinical trial requires ~5,000 doses (estimate)

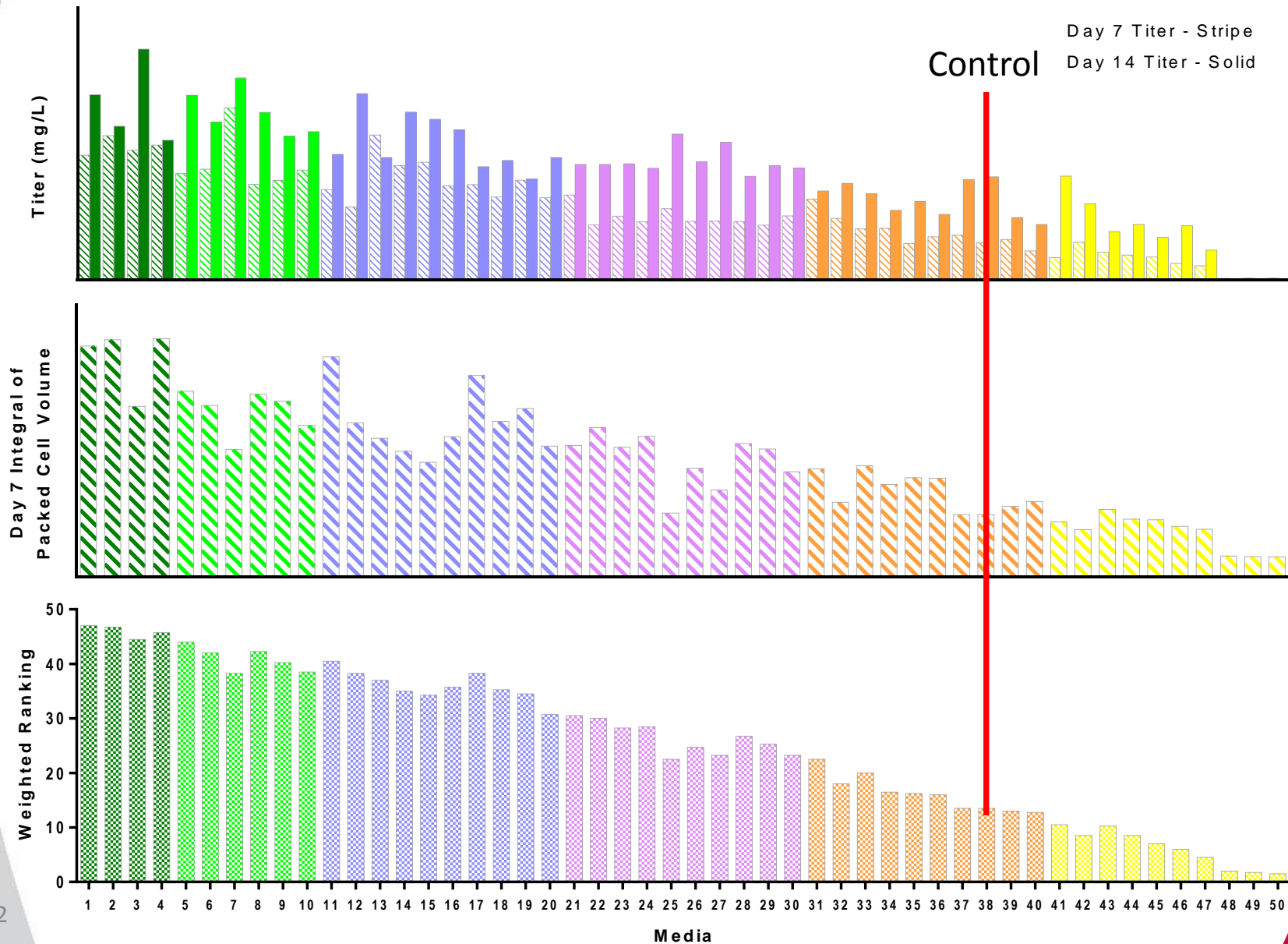
- To support Phase III and commercialization, the 2nd generation cell line was further optimized for higher yield
 - Process optimization involved
 - New media selection
 - New feed strategy
 - Optimized bioreactor conditions
 - Completely disposable inoculum
 - Produced in 1,000 L SUB
- How did we achieve this?



First Step: HTP Media Screening

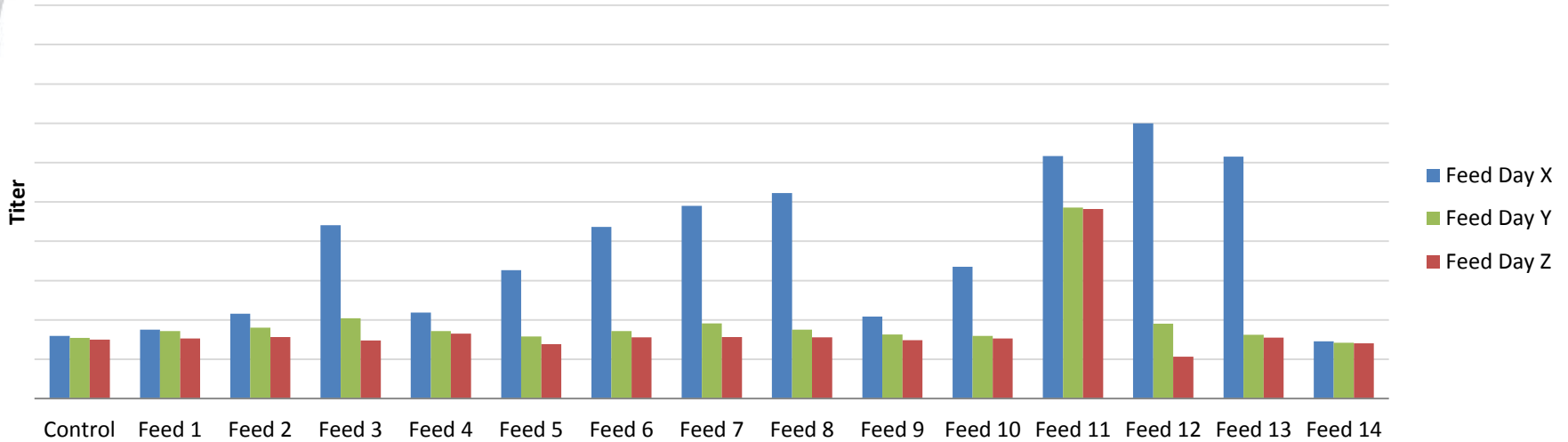
- Screened 50 different media in duplicate
- Checked Packed Cell Density (PCD; Day 0, 5, 7, and 10)
- Checked titer on Day 7 and 14
 - Protein A titer analysis by HPLC
- Created a ranking system based on PCD and titer
- Chose 3 media to move forward
 - Adaptation and larger growth studies in spinners
 - Test product characteristics
 - Selected 2 media for feed screen studies

1st Step: Media Screen

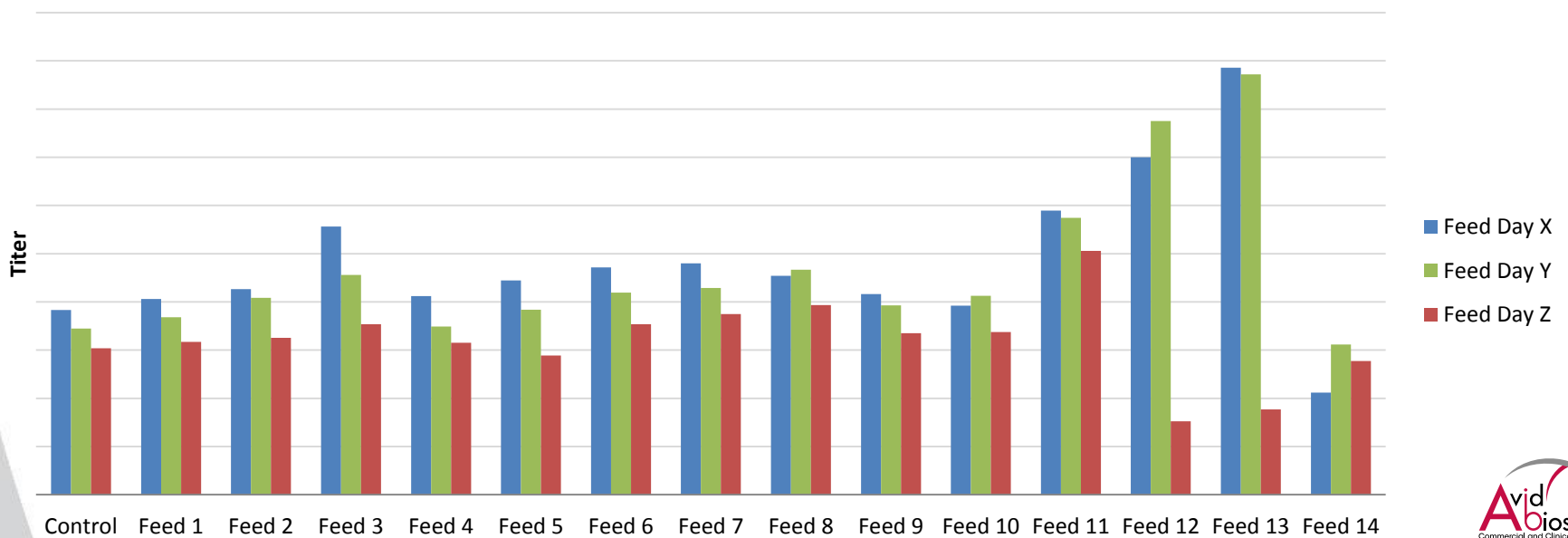


2nd Step: Feed Screen

Media 1 Feed Screen Titters



Media 2 Feed Screen Titters

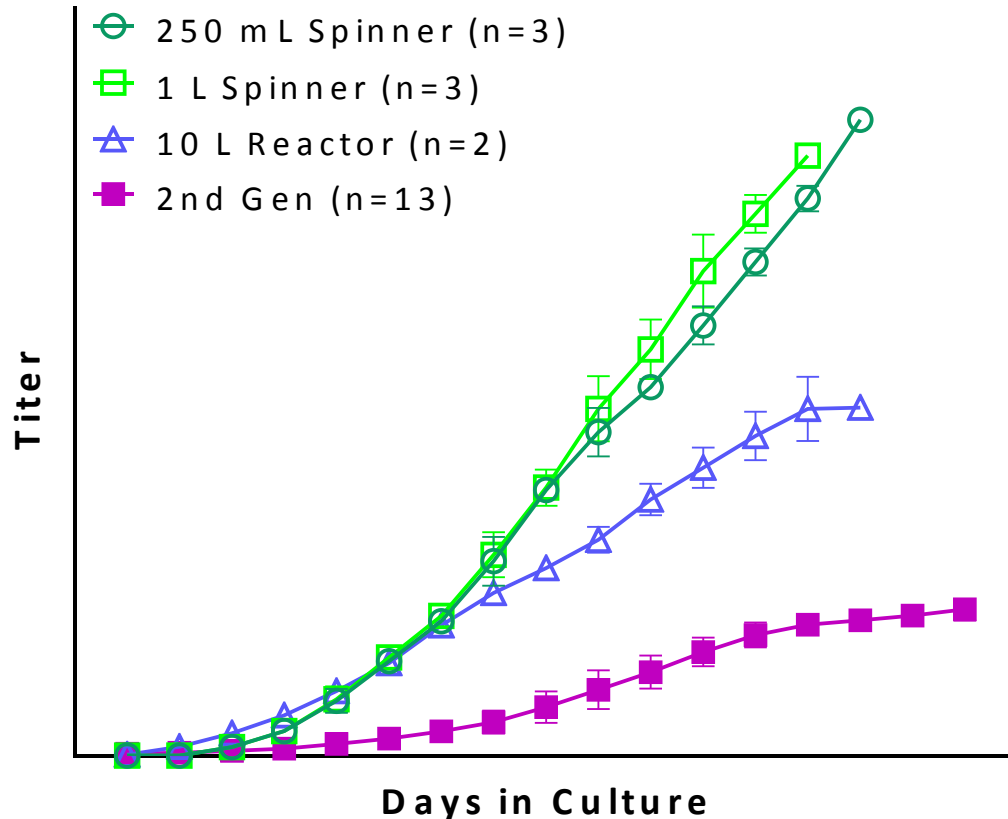


2nd Step: Characterization of Feed Screens

- Purified product from highest producing conditions
- Assays performed
 - Antigen Binding
 - Cation Exchange Chromatography
 - Size Exclusion Chromatography
 - Carbohydrate analysis
- Reduced to only 1 medium and tested combinations of the most effective feeds

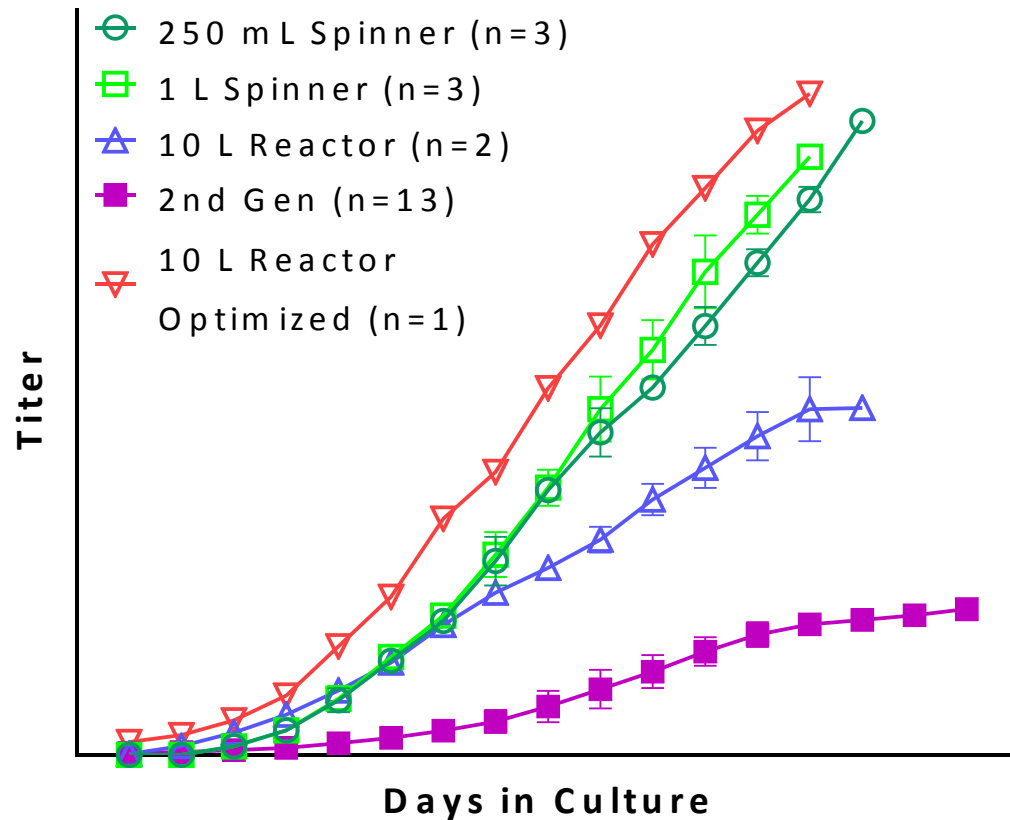
3rd Step: Combination Feeds/Multiple Days

- High Throughput Screening of multiple feeds over multiple days achieved our goal of a 4X increase in titer
- Moved on to small scale spinners followed by reactor trials

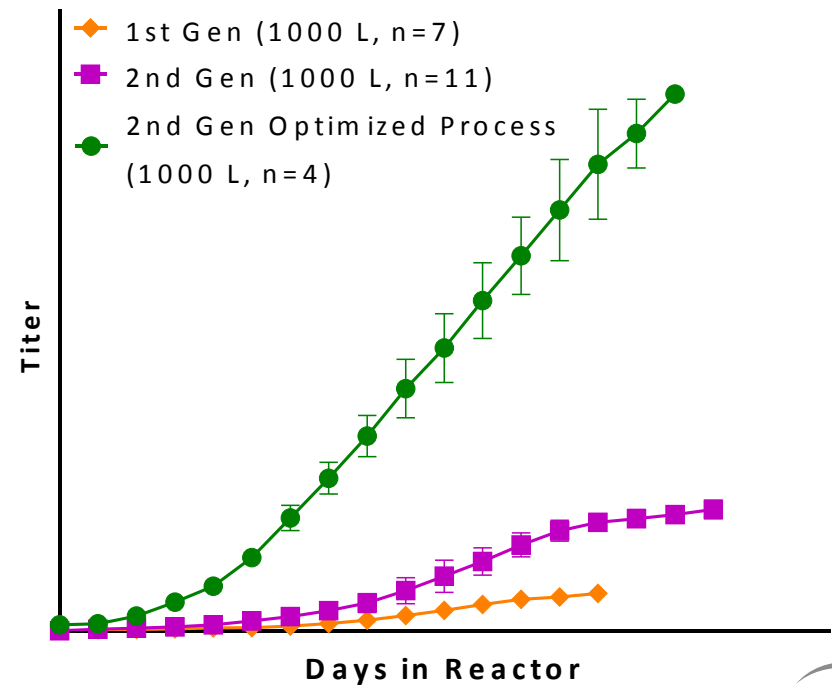
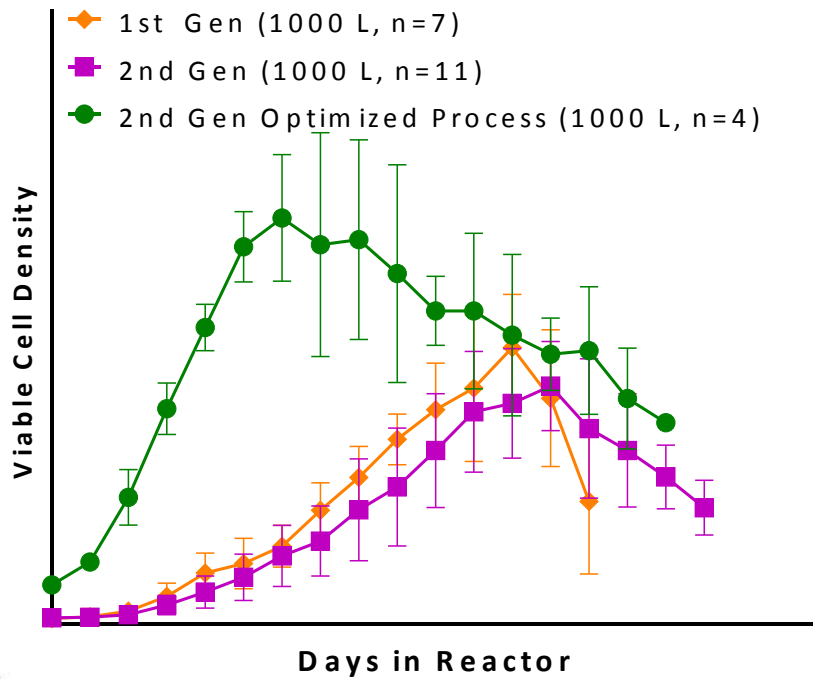
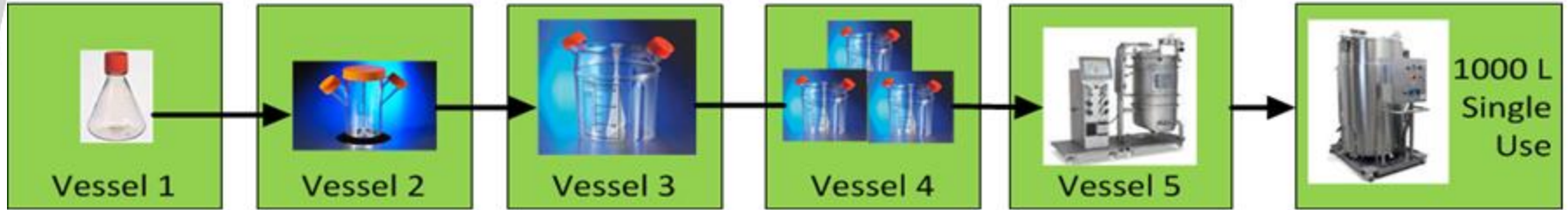


4th Step: Reactor Condition Optimization

- Inoculum density was evaluated
- Sparging strategy was evaluated
- Timing of the feeds were evaluated



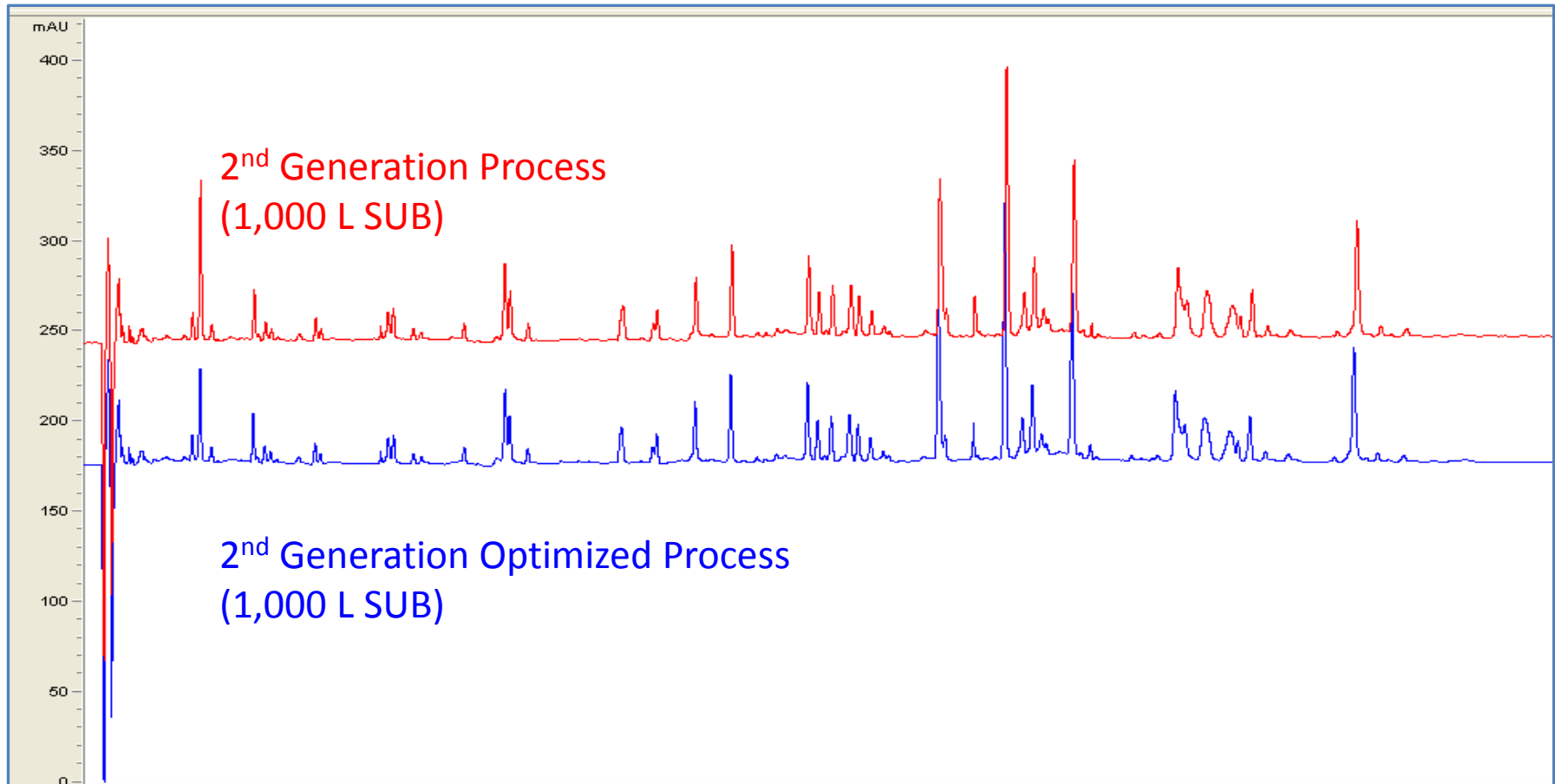
2nd Generation Optimized Process Performance



Product Comparability Demonstrated

Lot Release for Drug Substance/Drug Product		Additional Characterization
General Visual Inspection Color and Appearance Particulate Matter (DP) pH	Identity IEF/cIEF Peptide Map	Analytical Ultracentrifugation N-terminal Sequencing C-terminal Sequencing Monosaccharide Composition Neutral Sugar Assay
Strength and Potency Protein Content Antigen Binding Potency Bioassay	Process-Related Impurities (DS) Residual protein A Residual DNA HCPs	Stability Studies
Safety Endotoxin Bioburden	Purity SEC IEX SDS-PAGE SDS-CGE Oligosaccharide Analysis (DS)	Non-Clinical Pharmacokinetics in Small Animal Model

2nd Generation Product Comparability Peptide Map



Summary: Changes Made for Phase III Clinical Trials

- Process changes (new media, new feed strategy with optimized bioreactor conditions and completely disposable upstream process)
 - Resulted in **more than 4X increase** in production with a high degree of similarity in product quality attributes
- Complete data package submitted to FDA as an IND Amendment resulting in approval for these process changes

Summary: Multiple Process Changes During Clinical Development

Phase I

- 1st generation cell line, limited process development (300 L SSB)
- One 300 L batch resulted in ~150 clinical doses

Phase II

- Scaled up from 300 L to 1,000 L SSB (~500 doses)
- 2nd generation cell line with optimized downstream process
- Then 1,000 L SSB to 1,000 L SUB
- 3X increase in titer resulted in 1500+ clinical doses

Phase III

- 2nd generation cell line, optimized feed strategy (1,000 L SUB)
- > 4X increase in titer over 2nd Gen resulted in ~ 7000 clinical doses

➤ Product Comparability was demonstrated through analytical characterization package and small animal PK

Acknowledgement

- Our client and their regulatory group
- Robert Garnick
- ExcellGene
- Avid Process Sciences
- Avid Manufacturing
- Avid QC/QA
- Accounting (paying the bills)

Thank You!

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