CHO MEDIA PLATFORM FACILITATES INTEGRATED CELL LINE DEVELOPMENT AND MEDIA OPTIMIZATION

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Introduction

The development process for a biotherapeutic protein begins with generating a high-performing stable cell line which can be used for manufacturing. After identifying top clones, optimization of the culture media is essential to maximize performance. Together this labor-intensive work typically requires the longest timeframe during the development process and strategies to shorten and streamline this work are in demand. Here we present an accelerated approach which integrates cell line development with the media optimization by implementing a CHO media platform. For development of this integrated process, we used two therapeutic antibodies, an anti-CD20 and anti-HER2.

Methods

Stable Transfection

- GS-CHO host cell line (Horizon Discovery) adapted to BalanCD® CHO Growth A medium (Cat # 91128) with 4mM Glutamine. Cultured at 37°C, 5%$C0_2$ with 120rpm agitation
- Anti-CD20 and -HER2 antibody: heavy and light chain in pD2531 vector (ATUM)
- Cells transfected using FectoPro reagent (Polyplus) in BalanCD® Transfectory™ CHO medium (Cat# 91147). 48 hours post-transfection selection administered by changing media to BalanCD® CHO Growth A without Glutamine with MSX
- Viable cell density measured using Vi-Cell XR (Beckman Coulter)
- Antibody titers in supernatant measured using ForteBio Octet Qk^e with Protein A biosensors

Semi-Solid Cloning

- CloneMedia CHO Growth A with no Glutamine (Part# K8830, Molecular Devices)
- Stable transfected cells were seeded into 96wp on Day 0
- Images were captured using the CloneSelect Imager on Day 0, 1, 8, and 13

Platform Fed-Batch Strategy

- 30ml culture in BalanCD® CHO Growth A media in 125ml shake flasks
- Initial Seeding Density: 3x10^5 cells/ml
- Feed: BalanCD® CHO Feed 4
- Feed Schedule: 20% v/v over 4 events
- Temp shift to 33°C on Day 6
- Nutrients / metabolites measured by BioProfile FLEX (Nova Biomedical)
- Cultures were harvested when viability dropped below 70%

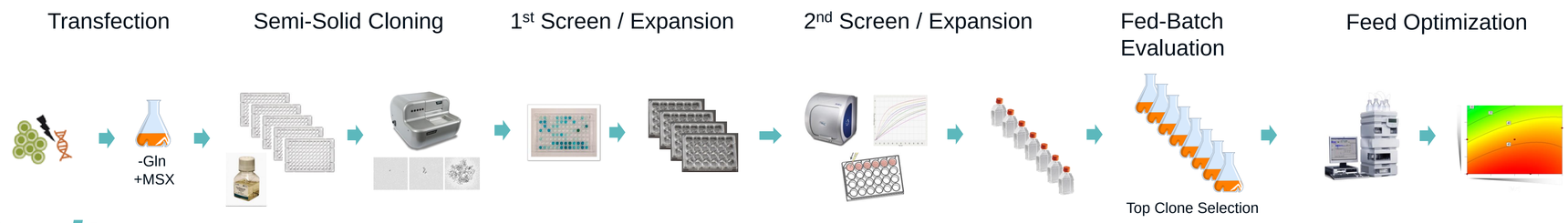
Antibody N-glycan Analysis

- Antibodies purified from clarified supernatant using Predictor MabSelect SuRe 96wp (GE)
- LabChip GXII (Perkin Elmer)
- Glycan Assay Release and Labeling Kit
- HT glycan LabChip reagent Kit

Feed Optimization

- Amino acids composition in spent media measured by HPLC (Agilent)
- Limiting amino acids in spent media were further enriched in new feed prototypes

Overview of Integrated Cell Line Development and Media Optimization Process



Results

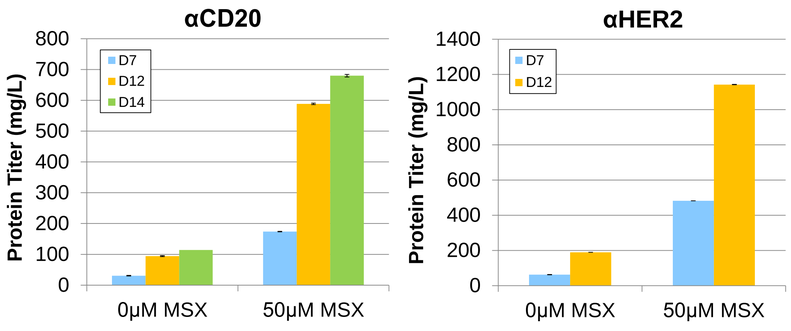
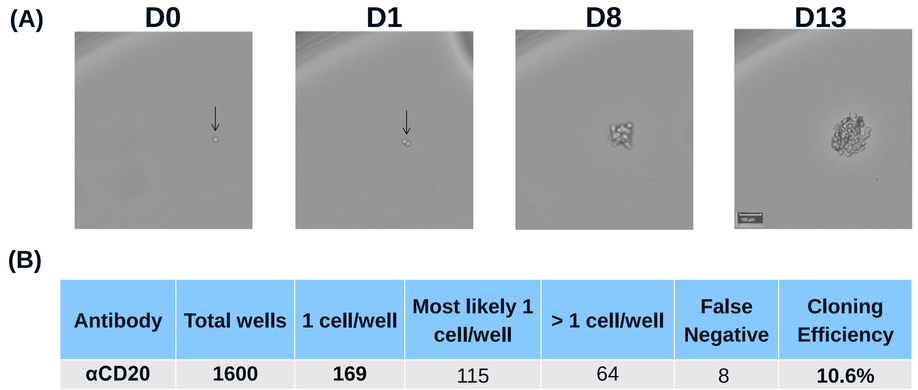


FIGURE 1. Stable Pool Fed-Batch Production. GS-CHO cells were transfected with αCD20 or αHER2 antibodies. After selection when cells recovered to >90% viability, a platform fed-batch production was performed and antibody titers measured (mean ± STD; n = 2)

➢ **MSX treatment during selection results in 5-6 fold increase in titer during fed-batch**



(B)

Antibody	Total wells	1 cell/well	Most likely 1 cell/well	> 1 cell/well	False Negative	Cloning Efficiency
αCD20	1600	169	115	64	8	10.6%
αHER2	1560	167	91	68	13	10.7%

FIGURE 2. Semi-Solid Cloning in 96wp. (A) Images of a single-cell per well colony for αHER2 (B) Summary of Cloning Results. Cloning Efficiency is the percentage of total wells which were 1 cell/well.

➢ **High-resolution images support evidence of monoclonality. Cloning efficiency was ~11% for both antibodies.**

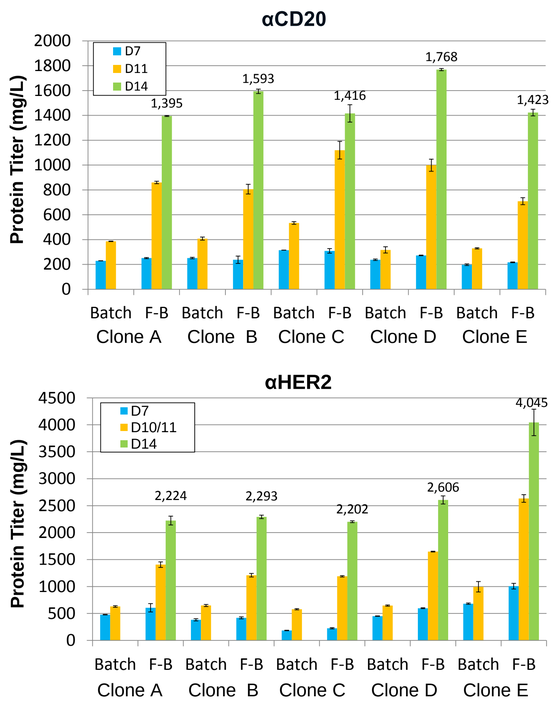


FIGURE 3. Fed Batch Production of Clones. Top clones were evaluated under platform fed-batch strategy. Antibody titers shown here (mean ± STD; n = 2)

➢ **Under platform fed-batch strategy, top αHER2 clones 2.2-4.0g/L and top αCD20 clones 1.4-1.8g/L**

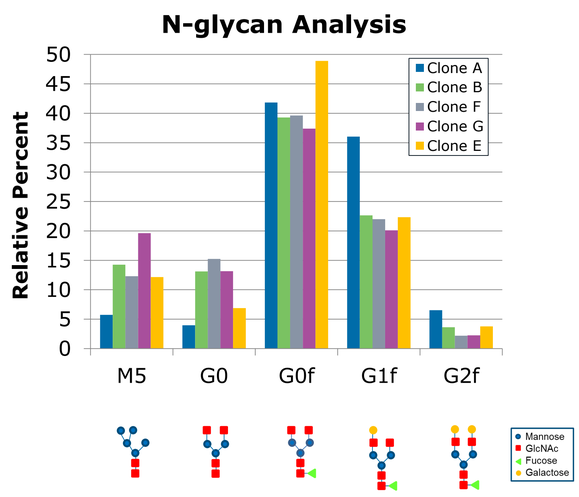


FIGURE 4. Product Quality Analysis: N-glycan for αHER2 Clones. Relative abundance of five major antibody N-glycans measured using LabChip GXII.

➢ **αHER2 clones show significant heterogeneity in N-glycan profile. Product quality can be part of clone selection criteria.**

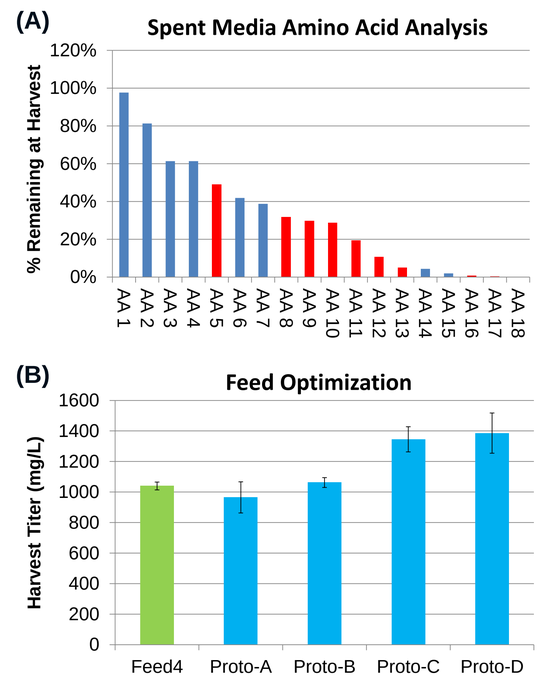
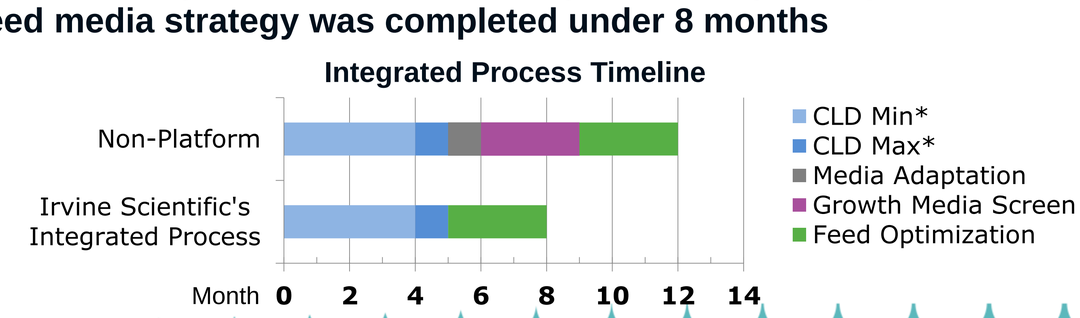


FIGURE 5. Feed Optimization.
(A) Spent media analysis determined percent remaining for each amino acid at harvest. Red bars denote key limiting amino acids (B) Fed-batch production of a αCD20 clone with 4 different prototype feed media (mean ± STD; n = 2)

➢ **Spent media analysis identified limiting amino acids during fed-batch. Prototype-D feed improved harvest titer by ~33%.**

Summary

- **Semi-solid cloning efficiency was ~11%, facilitating reasonable throughput while providing key imaging data for monoclonality**
- **Top five αHER2 clones achieved harvest titers between 2.2 - 4.0 g/L under platform fed-batch production in shake flasks, top αCD20 clones between 1.4 - 1.8 g/L**
- **Optimization of feed media further increased the harvest titer for a αCD20 clone by ~33%**
- **By performing cell line development in BalanCD® CHO Growth A media, we bypassed the need for media adaptation and growth media screening. This integrated process from DNA to top clones with optimized feed media strategy was completed under 8 months**



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