Journal of Bioanalysis & Biomedicine

Zhang and Hill, J Bioanal Biomed 2016, 8:2
DOI: 10.4172/1948-593X.1000148

Research Article Open Access

Overcome Challenges to Successful Manufacture of Biosimilars through Media and Feed Screening and Cell Culture Process Optimization

Min Zhang* and Timothy Hill

FUJIFILM Diosynth Biotechnologies, USA

Abstract

In the journey of drug development through regulatory approval, product quality attributes of a biosimilar protein must compare within defined limits to those of the innovator product. Unlike small molecule drugs, whose structure can usually be completely defined and entirely reproduced, biologicals are typically more complex and consist of heterogeneous populations not entirely identical to an innovator product. Therefore, biosimilarity is generally demonstrated as having matched product quality attributes, comparable *in vitro* biological activity, and no clinically meaningful differences between the biosimilar drug and innovator product.

The complexity of recombinant protein manufacturing processes, including expression systems (i.e., host cell line, expression vector, cell line development process), cell culture process conditions and related nutrient systems, such as cell culture media and feeds, present significant challenges to achieve the required product quality for biosimilars. To address these challenges, a systematic approach combining media toolbox methodology and bioprocess "know-how" has been developed to screen and optimize manufacturing conditions that promote the desired product quality profiles of recombinant proteins. Results using this strategy are presented to highlight the efficacy of this approach and successful implementation in manufacture of biosimilar recombinant monoclonal antibodies.

Keywords: Biosimilars; Manufacturing; Cell culture; Media toolbox; Bioprocess

Introduction

Biosimilars are biological products that are highly similar to regulatory approved biological products or "innovator" products, which are also known as reference products [1,2]. Biosimilars are generally defined as having no clinically meaningful differences in terms of safety and efficacy as compared to "innovator" products. Unlike the small molecule drugs, biologics generally exhibit high molecular complexity and are almost unlikely to be shown structurally identical to an innovator product. The molecular complexity includes protein primary amino acid sequence, post-translational modifications to protein or amino acids such as glycosylation, and higher order protein structure (protein folding and protein-protein interactions). In many cases, even minor structural differences can significantly affect a protein's safety and/or effectiveness.

Biosimilarity is defined as the biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components and that there are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency of the product. Comparative analytical data provide the foundation for a biosimilar product intended for regulatory submission [3]. Ensuring good biosimilarity requires consideration of a number of factors during manufacturing processes [4], of which the biologic expression system and biomanufacturing process present significant complexity and challenges. The process conditions can significantly affect the biosimilar product structure, influencing the biosimilarity. Therefore, the development of a suitable and robust manufacturing process with the appropriate control strategy is critical in biosimilar development [5,6].

To address these challenges, we have developed a systematic approach of combining cell culture process knowledge and media toolbox methodology to screen and optimize cell culture media and feeds, and the cell culture process conditions to promote the desired product quality profiles of recombinant proteins. The efficiency and efficacy of this development approach has been well demonstrated by a couple of case studies. The processes identified by using this strategy have been successful implemented in manufacturing of biosimilar recombinant monoclonal antibodies. Due to the complexity of biologic systems as describe above, no single set of conditions are expected to work consistently to achieve cell expression of protein products with desired biosimilarity. However, the strategy described has proven successful to narrow the study scope and provide guidance for biosimilar product development.

Materials and Methods

Bioreactor cell culture

Bioreactor systems at varied scales from multiple vendors were used for fed-batch cell culture studies, including BIOSTAT A+, BIOSTAT B (Sartorius AG) for 2 L scale, Xcellerex 10 L (XDR-10) and 200 L (XDR-200) (GE Healthcare). The systems consisted of a fully integrated gas mixing system, rotameter assembly or mass flow controller, pH control via CO_2 and base (1 N NaOH) addition, and dissolved oxygen (DO) control via air and O_2 valves. Seed cultures were expanded in shake flasks or bench-scale bioreactors and used to seed production bioreactors with working volumes varied by the process and bioreactors. In fed-batch production bioreactors, the process conditions including process temperature and temperature shift, and process pH were maintained according to the experiment design (pH ranged from pH 6.8 to pH 7.1; temperature ranged from 35°C to 37°C, and temperature shift occurred on cell culture day 6 from 37°C to 34°C) while DO was maintained at

***Corresponding authors:** Min Zhang, Upstream Process Development, FUJIFILM Diosynth Biotechnologies, 3000 Weston Parkway, Cary, NC 27519, USA, Tel: 919-388-5672; E-mail: min.e.zhang@fujifilm.com

Received April 07, 2016; **Accepted** April 22, 2016; **Published** April 29, 2016

Citation: Zhang M, Hill T (2016) Overcome Challenges to Successful Manufacture of Biosimilars through Media and Feed Screening and Cell Culture Process Optimization. J Bioanal Biomed 8: 023-027. doi:10.4172/1948-593X.1000148

40%-60% per specific process. The nutrient feeds were added as bolus additions according to the specific process needs. The glucose level was maintained >1.5 g/L with concentrated glucose feed (470 g/L). The cell culture duration was 14–17 days.

Cell lines

Chinese Hamster Overy (CHO) cell lines (DG44-derived and Glutamine Synthetase (GS)-derived) expressing monoclonal antibodies (mAbs) were used in the studies.

Cell culture analytic analysis

Viable cell density and cell viability were analyzed with Vi-CELL XR Cell Viability Analyzer (Beckman Coulter). Glucose, lactate, glutamine, and glutamate were analyzed by YSI 2700 Select Biochemistry Analyzer (YSI) or Cedex Bio HT analyzer (Roche), pH, pO_2 and pCO_2 concentrations were analyzed with a Bayer RapidLab 248 Blood Gas Analyzer (Bayer Healthcare). Ammonia was measure with Cedex Bio HT analyzer (Roche). Osmolality was analyzed with an Advanced Microsystems Osmometer Model # 3300 (Advanced Instruments, Inc.).

Product analysis

IgG titers of the cell culture time points were determined by using Poros A/20 4.6 × 50 mm columns (Applied Biosystems) on a Waters Acquity H-Class UPLC (Waters). The samples were injected neat and the concentration was calculated based off a calibration curve ranging from 5.0 to 120.0 µg.

N-linked glycans were analyzed by fluorescence detection HILIC UPLC (Waters). N-Glycans were released from Protein a purified antibody by using PNGaseF following desalting using a 50 kDa spin filter with water. Removal of protein was facilitated by ethanol precipitation, followed by formic acid treatment to fully reduce the N-terminus of the glycans. N-glycans were subsequently purified and labeled with 2-AB dye, followed by CU cartridge clean-up to remove excess dye. Identification of the glycans was performed by comparison against a 2-AB labeled Human IgG N-linked library from Prozyme, spiked with 2-AB labeled Man5 standard. The separation of glycans was performed using a Waters Acquity UPLC BEH Glycan, 1.7 µm, 2.1 × 150 mm column (P/N 186004742) held at 40°C on a Waters Acquity H-Class UPLC. A gradient of MPA (100 mM Ammonium Formate, pH 4.5) and MPB (ACN) was performed to separate the glycans using a change in MPB of 72% to 62% from 3.5 to 45 min.

ECI-MS N-linked glycan analysis was referred to the published method [7].

DOE statistical analysis

The DOE statistical study design and data analysis was performed by using JMP Design of Experiment software (SAS).

Results and Discussion

Product quality is cell line and process dependent

Product quality attributes including N-linked glycans of monoclonal antibody (mAb) can be significantly affected by cell line and cell culture process condition. During an upstream process development for a Biosimilar mAb, multiple CHO cell lines (clones) were selected through cell line engineering process. Those clones were further evaluated in cell culture process optimization studies in bioreactors, in which varied bioreactor process parameters were evaluated with DOE (Design of Experiment) statistical design. The protein A-purified antibody products were analyzed by ESI-MS to evaluate N-linked glycans (Figure 1). The Innovator mAb was used as reference material in the analytical test to show N-glycans comparability. With the same cell culture process condition, the mAb produced from CHO clone a showed similar glycan profile as compared to the innovator product, while the protein from clone B had quite different glycan profile (Figure 1A). Varied glycan profiles were observed in other selected clones (data not shown). To further improve cell culture performance including cell growth, titers, and product quality, a number of bioreactor process parameters were

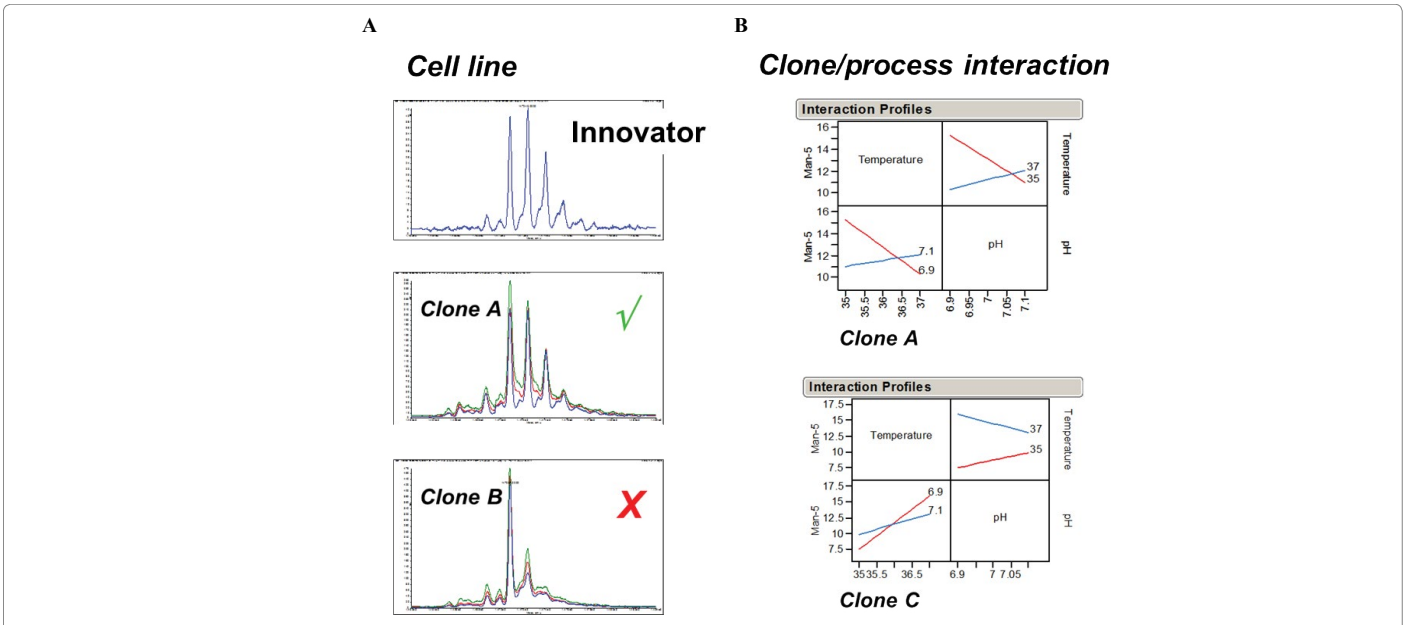

Figure 1: The impact by upstream bioprocess on N-Linked Glycans. **A)** ESI-MS profiles to show the impact by cell lines. The cell culture was maintained at 37°C, pH 7.0 and 50% DO (dissolved oxygen). The bioreactor samples at varied time points are color-coded, blue-day 12 sample, red-day 14 sample, green-day 17 sample; **B)** JMP DOE profiles to show varied interaction between cell line (clone) and cell culture process parameters that were varied by DOE conditions (pH 6.9 to pH 7.1, temperature 35°C-37°C).

optimized in a series of bioreactor studies with DOE design. Figure 1B illustrates a DOE statistical model on Mannose-5 (Man-5) glycan responding to the changes of process pH and temperature with two different clones. The DOE model clearly illustrates an opposite response to these two process parameters between the two CHO clones. For instance, at pH 6.9, the Man-5 from clone A showed a decreasing trend with increasing temperature, whereas it the opposite trend was observed with clone C.

From these studies, we demonstrated that the mAb product quality (e.g. N-linked glycans) was significantly affected by CHO cell lines and cell culture process conditions. This demonstrates the great complexity of the product quality control of Biosimilars. It also provides opportunities to produce biosimilar products with desired product quality by optimizing upstream process conditions.

Cell culture process conditions and nutrient supplement affect N-linked glycans

To further improve product quality of this biosimilar mAb to make it match with innovator mAb as close as possible, detailed cell culture process optimization studies including bioreactor condition optimization and cell culture supplements were performed to the selected CHO cell lines. A temperature shift was incorporated in a bioreactor study, in which the bioreactor temperature was shifted from 37°C to 34°C on day 6. In the ESI-MS profile of the protein at harvest, the temperature shift did not show the improvement on N-linked glycans while the constant bioreactor temperature supported better glycosylation as compared to the innovator product (Figure 2A). Varied process pH was evaluated in another bioreactor study. Figure 2B illustrates the N-linked glycan profiles of the mAb at harvest, it appeared that the neutral process pH benefited the small glycan species including Man-5 (highlighted with red arrows) compared to the slightly acidic process.

Besides the cell culture process, the complexity of cell culture media chemistry in basal media and feed media represents another challenge to biotherapeutics production, not only to sustain good cell culture performance including cell growth and protein productivity but to maintain desired product quality. It's not uncommon to see the cell culture nutritional supplements significantly affect the product quality [8,9] In addition to bioreactor process parameters, a sugar supplement was evaluated in the bioreactors. As compared to the condition without sugar supplementation, the sugar addition significantly reduced the small glycan species which made them look comparable to the innovator product (Figure 2C).

Upon conclusion of cell culture process development for this biosimilar mAb, and after evaluation of the bioreactor process conditions and media supplements, we were able to identify an optimal cell culture process condition to produce antibody from CHO clone B with similar, acceptable product quality attributes as compared to innovator product. Figure 2D illustrates the N-linked glycan profile improvement of the antibody produced by clone B by implementing

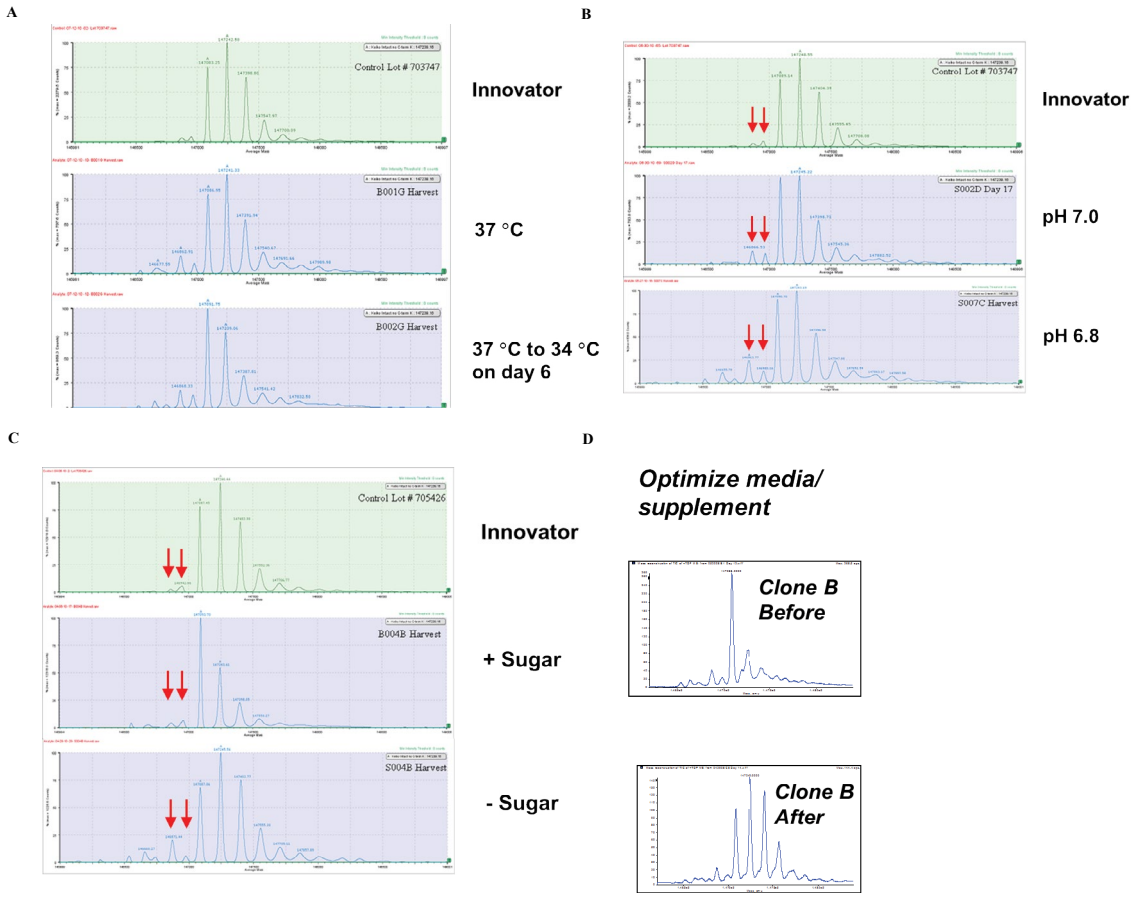

Figure 2. The impact by cell culture process parameters and media supplement on N-Linked Glycans. **A)** Impact by temperature. The cell culture was maintained at pH 7.0, 50% DO, and varied temperatures; **B)** Impact by pH. The cell culture was maintained at 35°C, 50% DO, and varied pHs; **C)** Impact by sugar supplement. The cell culture was maintained 37°C, 50% DO, and pH 6.9; **D)** Optimized glycan profile after cell culture process optimization.

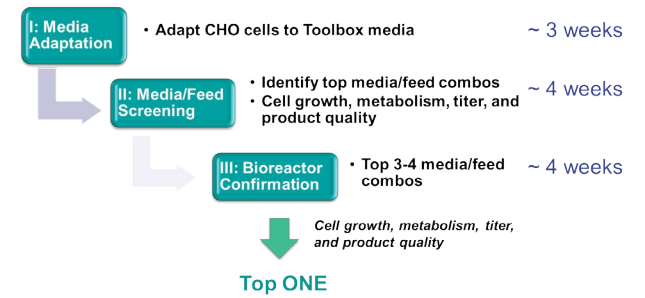

Figure 3: Schematic representation of media toolbox implementation at FDBU.

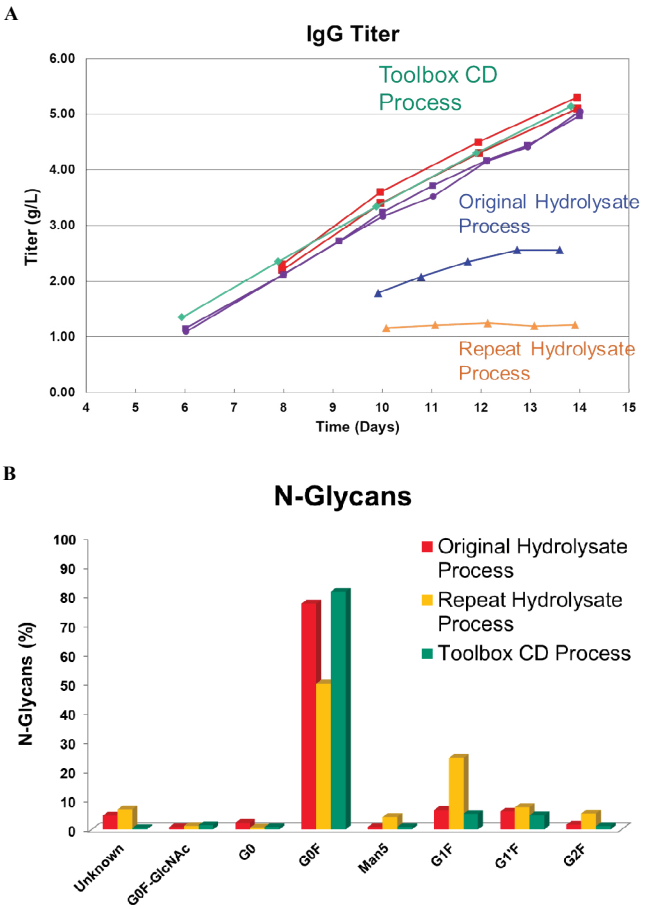

Figure 4: A mAb case study to show the efficacy of media toolbox for process improvement to achieve desired product performance. **A)** IgG titer. The different bioreactor scales were color-coded: red - 2 × 2 L bioreactor; purple – 2 × 10 L bioreactors; green – 1 × 200 L bioreactor; **B)** N-linked Glycans.

the optimized process condition. The process knowledge learning from this mAb has been leveraged with other proteins expressed in the CHO cell culture.

Cell culture media toolbox strategy

In addition to using process knowledge to achieve desired biosimilar proteins, we also implemented a "Media Toolbox" approach to help control the product quality of antibody products within the acceptable ranges. Our media toolbox is a collection of 4 to 5 high performing cell culture basal and 4 to 5 feed media used for screening diverse CHO cell lines to rapidly deliver desired cell culture performance. The media toolbox was established by screening a variety of off-the-shelf and prototype cell culture basal and feed media from 6 media vendors (including 63 basal media, and 19 feed media) with more than 15 CHO cell lines covering varied CHO subtypes (CHO-DG44, CHO-DXB11, GS-CHO, CHO-S) in over 25 cell culture experiments.

Figure 3 shows a schematic strategy of media toolbox implementation. To ensure representative cell culture results, the CHO cells are first adapted from original media into the toolbox media with minimal 5 passages of subculture. After media adaptation, a screening study in shake flask is performed to identify the top toolbox media/feed combinations by evaluating the cell culture performance including cell growth, key cell culture metabolites, protein titer, and product quality. Based on the performance results, a few top media/feed combinations are tested in bioreactors to select the final media/feed combination for optimization studies as described above.

A case study: Application of media toolbox

A case study is described here to show the efficacy of media toolbox strategy. A cell culture process was developed for a monoclonal antibody in 2007 with a media containing soy hydrolysates that produced about 2.5 g/L of the mAb at harvest (Figure 4). In 2014, when the process was run again, we were not able to reproduce the mAb titer and N-linked glycan profile of the mAb from the original production process. Possible reasons for these unexpected results are compositional changes in the hydrolysate preparation and/or changes to the feed media as this media had been discontinued by the vendor and required custom preparation.

To overcome these challenges and especially to address the product quality change, we implemented the media toolbox screening (Figure 3). After media adaption and shake flask screening studies (data not shown), one media and feed combination was selected based on the titer and glycosylation profiles of the mAb for bioreactor studies. As compared to the original hydrolysate process, the process with the toolbox chemically-defined media resulted in mAb titer of approximately 5 g/L on harvest day 14, which was twice the previous titer (Figure 4A). Most importantly, after application of the new media/feed, the N-linked glycan profile was comparable to the reference material derived from the original hydrolysate process (Figure 4B). The process has been scaled from 2 L to 10 L to 1,000 L bioreactor scale while maintaining product titers and product quality throughout demonstrating the robustness of the toolbox media process.

Two research and development scenarios for biotherapeutics process development and optimization at FDB

To achieve specific product quality requirements for biotherapeutic proteins including biosimilars, during clinical development, we have established two unique but closely related development approaches (Figure 5). Whether cell lines are developed internally using Fujifilm's Apollo™ system or developed externally, both toolbox media screening and process optimization were found to be effective for generating biosimilars that match key innovator molecule attributes and activity profiles.

In late 2014, a CHO-DG44 based cell line development (CLD) platform (Apollo™) was established at FDB to support our client projects. The Apollo™ platform consists of a proprietary CHO host cell line, expression vector, cell culture media and feed, and a platform cell culture process. Apollo™ enables projects to start from a gene sequence and progressing through cell line development, a series of bioreactor screening and optimizations to identify a cell culture process

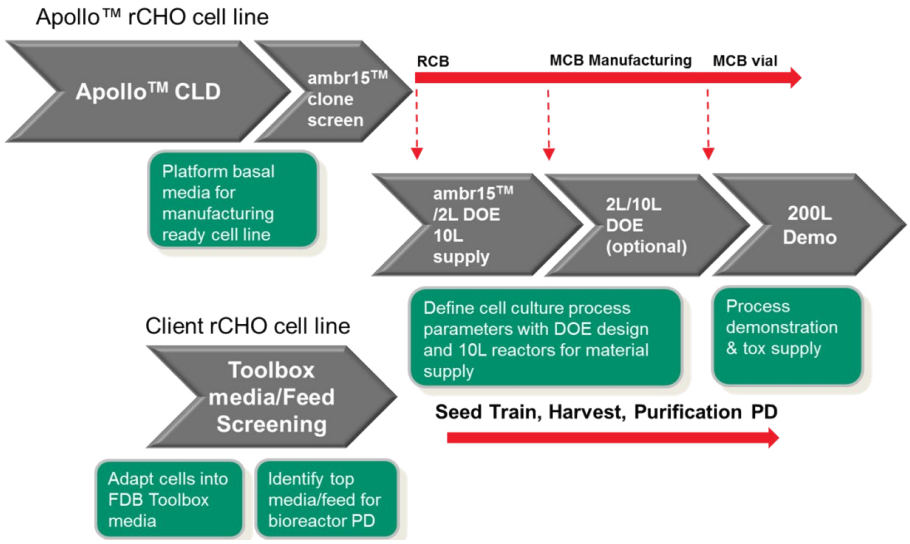

Figure 5: Streamlined CHO cell culture process flow with three development scenarios at FDBU.

demonstrated at varied bioreactor scales including ambr15^{TM} micro-bioreactor and 2 L, 10 L, and/or 200 L process development bioreactors. Starting with an existing cell line, we would start with implementation of the media toolbox, as described above.

In conclusion, to address and overcome challenges to manufacture biotherapeutic products including biosimilars, FDB has established a systematic strategy by combining media toolbox methodology and bioprocess "know-how" to achieve the desired product quality profiles of recombinant proteins. The efficiency and efficacy of this approach has been demonstrated in a number of CHO cell culture programs in manufacture of recombinant monoclonal antibodies. Regardless which approach is chosen for biotherapeutic clinical development, as the linkage between all three scenarios at FDB, the cell culture process knowledge or bioprocess "know-how" connects all as a systematic strategy to promote a suitable bioprocess to generate desired product quality for recombinant proteins including biosimilars.

Acknowledgement

Authors are grateful to the following people who contributed to this work at Fujifilm Diosynth Biotechnologies (FDB): Upstream Process Development - Carrie Weymer, Leisha Youngblood, Stewart McNaull, Gian Arias; Downstream Process Development - Michael Murray, Philip Ropp; Analytical Development - Clara Smith, Greg Adams.

References

1. Mayden KD, Larson P, Geiger D, Watson H (2015) Biosimilars in the United States: Considerations for Oncology Advanced Practitioners. J Adv Pract Oncol 6: 108-116.
2. Braun J, Kudrin A (2015) Progress in biosimilar monoclonal antibody development: the infliximab biosimilar CT-P13 in the treatment of rheumatic diseases. Immunotherapy 7: 73-87.
3. FDA Guidance (2015) Scientific considerations in demonstrating biosimilarity to a reference product – guidance for industry.
4. FDA Guidance (2015) Quality considerations in demonstrating biosimilarity of a therapeutic protein product to a reference product – guidance for industry.
5. Declerck P, Farouk-Rezk M, Rudd PM (2016) Biosimilarity Versus Manufacturing Change: Two Distinct Concepts. Pharm Res 33: 261-268.
6. Lee JF, Litten JB, Grampp G (2012) Comparability and biosimilarity: considerations for the healthcare provider. Curr Med Res Opin 28: 1053-1058.
7. Stadlmann J, Pabst M, Kolarich D, Kunert R, Altmann F (2008) Analysis of immunoglobulin glycosylation by LC-ESI-MS of glycopeptides and oligosaccharides. Proteomics 8: 2858- 2871.
8. Li F, Vijayasankaran N, Shen AY, Kiss R, Amanullah A (2010) Cell culture processes for monoclonal antibody production. MAbs 2: 466-477.
9. Gramer MJ (2014) Product quality considerations for mammalian cell culture process development and manufacturing. Adv Biochem Eng Biotechnol 139: 123-166.