Exploration of non-originator NISTmAB generated from NS0 clones and cNISTmAb generated from NISTCHO

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Abstract

The current regulatory guidance requires comparability assessment to support major manufacturing process changes during the development or post-approval for therapeutic protein products. A hierarchal risk-based approach is recommended at all stages of development, starting with analytical testing to ensure quality and biological characterization to ensure potency, and if needed, in vivo pharmacokinetic or pharmacokinetic-pharmacodynamic studies, clinical safety and/or efficacy studies. In 2016, NIST the first reference material for a monoclonal antibody, NIST RM 8671, also known as NISTmAb, which was produced by its originator using a proprietary cell line and process. The introduction of NISTmAb provided a standard material that could be shared throughout the biopharmaceutical industry for the qualification and validation of methods used to characterize product quality through process and product development. In 2018, NIST announced that they had developed three non-originator NS0 expression cell lines (NS0-59, NS0-60, and NS0-66) that produced NISTmAb that embodied quality attributes with a sufficient degree of sameness to the NIST RM 8671. In early 2023, NIST released NISTCHO test material (RGTM 10197) that has been engineered to express a non-originator version of NISTmAb (cNISTmAb). Here we show our efforts to culture the NISTCHO cell line under tightly controlled bioreactor conditions. This work will provide the foundation for case studies of cell substrate manufacturing changes and adds to the discussion of regulatory considerations to ensure comparability of therapeutic protein drug products post cell line change.

Introduction

The therapeutic protein and monoclonal antibody products assessed within the Office of Biotechnology Products are manufactured using cellular expression systems called cell substrates. A manufacturing process change that involves the introduction of a new cell substrate is considered a high risk change that necessitates a comparability exercise to provide assurance of safety and efficacy of the protein therapeutic produced from the new cell substrate. Examples of cell substrate changes submitted to the agency include adaptation to serum-free media, selection of a new clone from the original host cell transfection, and transition to an entirely new, higher productivity cell line utilizing a new expression vector(s). These manufacturing changes have generated challenging regulatory questions for the entire assessment team because changes to the cell substrate have the potential to impact therapeutic protein structure, potency, and/or impurity profiles, which in turn may or may not impact pharmacokinetics (PK), pharmacodynamics (PD), clinical safety and/or efficacy. When Sponsors submit questions, IND amendments or prior-approval supplements for manufacturing changes to cell substrates, gathering information on past precedent and achieving consensus about what supporting data must be provided has been a challenge. The current recommendation is a hierarchal approach in which the Sponsor would start with analytical testing to ensure quality, followed by biological characterization and then, if needed, follow with pharmacokinetic, pharmacodynamic, and/or clinical safety and/or efficacy studies. To develop understanding of the impact of cell substrate on specific critical quality attributes of monoclonal antibody products, we will generate non-originator NISTmAb from CHO and NS0 under controlled bioreactor conditions and perform analytical characterization to determine which quality attributes are most impacted. In this poster we show preliminary data from the NISTCHO RGTM 10197 in which we generated a passage 9 working cell bank (WCB) from the passage 6 master cell bank (MCB) and tested growth and productivity in several media types in shake flasks. Future studies will optimize bioreactor process conditions for growth of NISTCHO and production of cNISTmAb, optimize process conditions for NIST NS0 clones for production of non-originator NISTmAb , and analytical characterization of the non-originator NISTmAb produced in CHO and NS0 with respect to the NISTmAb RM.

Materials and Methods

NISTCHO Research-Grade Test Material (RGTM) 10197 was obtained as one vial of cryopreserved Chinese hamster ovary K1 subtype (Sp: *Critelus griseus*; CHO-K1) lineage cell suspension with a nominal cell number of 1.3 x 10^7 cells and a time-in-culture post-clonal isolation of 6 passages. The vial was thawed and propagated using a protocol adapted by NIST from the Sigma-Aldrich CHOZN® Platform technical Bulletin to generate a passage 9 post-clonal isolation working cell bank (WCB; 10197/p09/EXCELL® CD CHO/FDA) as instructed in the NISTCHO test Material Guidance Document (DOI January 6, 2023). A vial of WCB was thawed and NISTCHO was further cultured in shake flasks in EXCELL® Advanced CHO Fed-batch Medium (with or without and EXCELL® Advanced CHO Feed 1 without glucose) and EXCELL® Advanced CHO HD Perfusion Medium. Samples were taken either daily or every other day for cell and nutrient analysis on the Bioprofile Flex2 (Nova Biomedical) and additional samples were collected for titer analysis using biolayer interferometry (Octet N1; Sartorius) equipped with Protein A sensors .

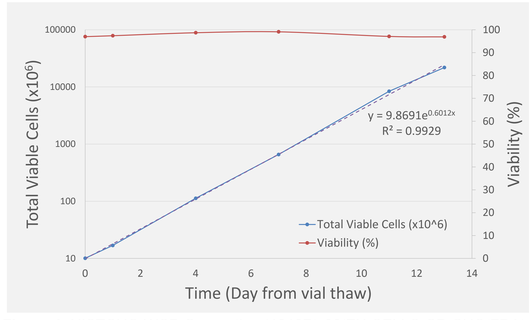

Figure 1. NISTCHO WCB Generation (10197/p09/EX-CELL® CD CHO/FDA) from MCB (10197/p06/EX-CELL® CD CHO/NIST). Total viable cells (blue line) and cell viability (red line) are shown with respect to time (day from vial thaw. Cell growth followed an exponential trend (R^2=0.99)

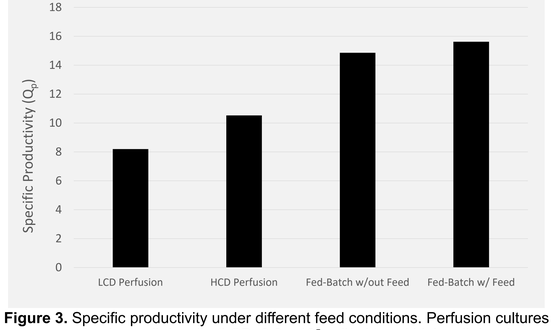

Figure 3. Specific productivity under different feed conditions. Perfusion cultures were run using low cell density (LCD; 8.8 x 10^5 cell/mL) and high cell density (HCD; 4.8 x 10^6 cells/mL) day 0 conditions, while fed-batch cultures were both seeded to 1.75 x 10^6 cells/mL on day 0.

Results and Discussion

We have to generated a passage 9 post-clonal isolation WCB (10197/p09/EXCELL® CD CHO/FDA) as instructed in the NISTCHO test Material Guidance Document. The NISTCHO cells remained in exponential growth during scale-up (Figure 1) with a viability above 95% and were in the mid-exponential growth phase when the WCB was cryopreserved. We cultured NISTCHO in shake flasks in EXCELL® Advanced CHO Fed-batch Medium, adding glucose up to approximately 5 g/L on days 3 and 5. We also added 5% EXCELL® Advanced CHO Feed 1 on days 3 and 5 to one flask to see if the proprietary feed resulted in increased growth, higher viability, and/or higher titer. We found that providing the NISTCHO culture the feed in addition to the glucose resulted in a higher maximum VCD between days 4-7 (12.5 x 10^6 versus 9.5 x 10^6 cells/mL) and a higher viability on day 7 (93.3% versus 83.0%) (Figure 2). However, the Feed did not significantly impact specific productivity (Figure 3). Since we intend to run NISTCHO in perfusion operation 5L glass benchtop bioreactors, we also assessed NISTCHO's growth and specific productivity in EXCELL® Advanced CHO HD Perfusion Medium. We found that NISTCHO grows at a faster rate in the perfusion medium (Figure 2) but the specific productivity is lower than in fed-batch medium (Figure 3). We also were able to determine that NISTCHO can be cultured up to 23.5 x 10^6 cells/mL in a shake flask while maintaining >97% viability in the perfusion medium (Figure 2). While we have cultured NISTCHO in both Ambr250 modular single-use stirred tank bioreactors and 5L glass benchtop stirred-tank bioreactors (Figure 4; data not shown), the process parameters are still being optimized to achieve the greatest cell growth, viability and productivity.

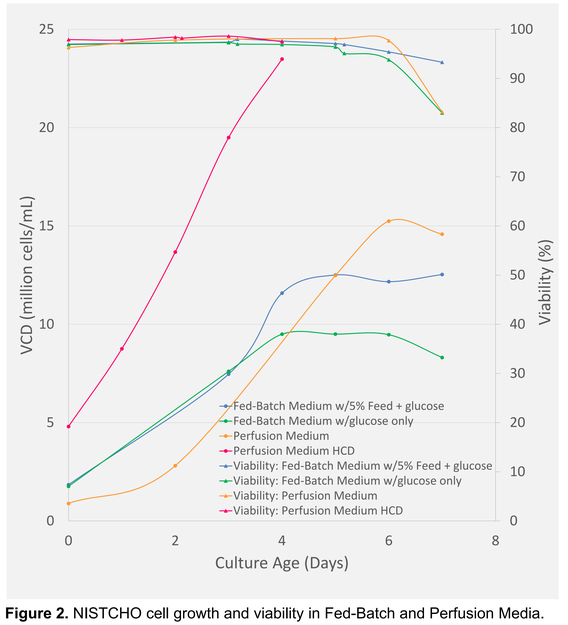

Figure 2. NISTCHO cell growth and viability in Fed-Batch and Perfusion Media.

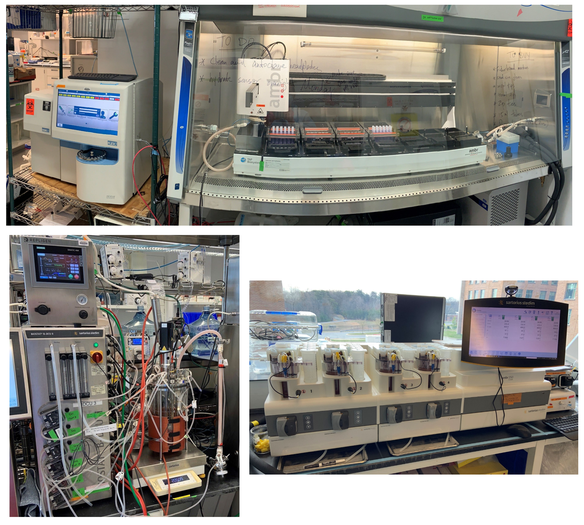

Figure 4. Stirred-tank bioreactor (STR) systems to be used to optimize NISTCHO and NIST NS0 process conditions and generate non-originator NISTmAb for analytical characterization. The top image is of our microbioreactor system integrated with a sampling module for the cell and nutrient analyzer that we will use to run DOEs to find optimal process conditions for the NISTCHO and NIST NS0 cell lines. The bottom left is one of our benchtop STRs operating with an ATF device for perfusion. The bottom right is our single-use modular bioreactor system used for scaling up processes selected from our microbioreactor runs.

Conclusion

This work is the start of optimization of growth conditions for NISTCHO being used to produce the optimal process conditions in stirred-tank bioreactors. We will also perform similar experiments in shake flask for the NS0 clones. Future studies will optimize bioreactor process conditions for growth of NISTCHO and production of cNISTmAb, optimize process conditions for NIST NS0 clones for production of non-originator NISTmAb , and analytical characterization of the non-originator NISTmAb produced in CHO and NS0 with respect to the NISTmAb RM.

Disclaimer

The opinions discussed in this poster are those of the authors and do not necessarily reflect FDA policy.