Received: 10 August 2021 | Revised: 12 October 2021 | Accepted: 13 November 2021

DOI: 10.1002/bit.27995

COMMUNICATION

BIOTECHNOLOGY AND BIOENGINEERING WILEY

Rapid cGMP manufacturing of COVID-19 monoclonal antibody using stable CHO cell pools

Rita Agostinetto[1] | Mara Rossi[1] | Jessica Dawson[2] | Angela Lim[2] | Mirva H. Simoneau[3] | Cyril Boucher[3] | Bernhard Valldorf[4] | Adin Ross-Gillespie[3] | Joseph G. Jardine[5,6,7] | Devin Sok[5,6,7] | Dennis R. Burton[5,7,8] | Thomas Hassell[6] | Hervé Broly[3] | Wolf Palinsky[3] | Philippe Dupraz[3] | Mark Feinberg[6] | Antu K. Dey[6]

[1]MerckSerono S.p.A, Guidonia di Montecello, Italy

[2]EMD Serono, Billerica, Massachusetts, USA

[3]Ares Trading SA/Merck SA Switzerland, Aubonne, Switzerland

[4]Merck KGaA, Darmstadt, Germany

[5]Department of Immunology and Microbiology, The Scripps Research Institute, La Jolla, California, USA

[6]IAVI, New York, New York, USA

[7]IAVI, Neutralizing Antibody Center, The Scripps Research Institute, La Jolla, California, USA

[8]Ragon Institute of MGH, MIT and Harvard, Cambridge, Massachusetts, USA

Correspondence

Antu K. Dey, IAVI, 125 Broad St, New York, NY, USA,
Email: adey@greenlightbio.com

Present address

Antu K. Dey, GreenLight Biosciences Inc., 200 Boston Avenue, Suite 1000, Medford, Massachusetts, USA.

Abstract

Therapeutic proteins, including monoclonal antibodies, are typically manufactured using clonally derived, stable host cell lines, since consistent and predictable cell culture performance is highly desirable. However, selecting and preparing banks of stable clones takes considerable time, which inevitably extends overall development timelines for new therapeutics by delaying the start of subsequent activities, such as the scale-up of manufacturing processes. In the context of the coronavirus disease 2019 (COVID-19) pandemic, with its intense pressure for accelerated development strategies, we used a novel transposon-based Leap-In Transposase® system to rapidly generate high-titer stable pools and then used them directly for large scale-manufacturing of an anti-severe acute respiratory syndrome coronavirus 2 monoclonal antibody under cGMP. We performed the safety testing of our non-clonal cell bank, then used it to produce material at a 200L-scale for preclinical safety studies and formulation development work, and thereafter at 2000L scale for supply of material for a Phase 1 clinical trial. Testing demonstrated the comparability of critical product qualities between the two scales and, more importantly, that our final clinical trial product met all pre-set product quality specifications. The above expediated approach provided clinical trial material within 4.5 months, in comparison to 12–14 months for production of clinical trial material via the conventional approach.

KEYWORDS

cGMP manufacturing, CHO pools, COVID-19, monoclonal antibody

Communication To The Editor

Multiple cell line sources are used as hosts to produce recombinant prophylactic and therapeutic proteins for human use. Recombinant Chinese hamster ovary (CHO) cell lines (Puck et al., 1958) remain a preferred host due to the reliability, robustness, and maturity of the technology in generating clonally derived cell line. Despite the high level of production, batch-to-batch consistency and robustness of a clonally derived CHO cell line, the approach is challenging due to the burden of time and resources required for stable clone isolation, selection, and provision of tested cell banks for cGMP manufacturing. Therefore, in recent past, several groups have reviewed their cell line development strategy to expedite entry into clinic (Scarcelli et al., 2017; Stuible

et al., 2018; Wright et al., 2017; Zhang et al., 2021) by generating comparison between non-clonal and clonal CHO cell produced materials (Fan et al., 2017), and accelerating IND-enabling Toxicology studies by using materials produced from non-clonal CHO cell lines (Bolisetty et al., 2020; Hu et al., 2017; Munro et al., 2017; Rajendra, Balasubramanian, Peery, et al., 2017). In addition to expediting the Toxicology study using materials from non-clonal CHO cell pools in non-cGMP production, as others have shown (Bolisetty et al., 2020; Hu et al., 2017; Munro et al., 2017; Rajendra, Balasubramanian, McCracken, et al., 2017), we wanted to evaluate the possibility of using non-clonal stable CHO cell pools to expediate production of a IgG1 monoclonal antibody (mAb) against severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), CC6.35, particularly through cGMP manufacturing of a single batch of material for a Phase 1 clinical trial. Since this approach (of producing material for early clinical studies using non-clonal CHO cell pools) has not been rigorously tested for scaled-up cGMP manufacturing and concerns remain that the cellular and genetic heterogeneity of non-clonal stable CHO cell pools may result in production variability and concomitant heterogeneous product qualities between batches, we present a case study wherein we used non-clonal qualified cell banks and platform processes to accelerate manufacturing of CC6.35 mAb. To do so, we used the novel transposon-based Leap-In Transposase® system (Rajendra, Balasubramanian, Peery, et al., 2017; Rajendran et al., 2021) for the development of stable CHO cell lines. The codon-optimized DNA sequence encoding the amino acid sequence for the Heavy chain (HC) and Light chain (LC) of CC6.35 mAb, along with corresponding signal peptide and the novel expression constructs based on the Leap-In transposon® system, were designed and synthesized. These synthesized DNA constructs along with transposase mRNA (Rajendran et al., 2021; Wilson et al., 2007) were used to co-transfect HD-BIOP3 glutamine synthase (GS) knock-out CHO-K1 host cells. Two promoter components were used to generate two unique sets of CHO cell pools: one using the EF1 promoter and another using the CMV promoter. Post-transfection the recovery of these two CHO cell pools was performed; after the initial recovery phase, the positive CHO cell pools were selected by outgrowth in a glutamine-free formulation at 37°C in 5% CO_2 and 70%–80% relative humidity and expanded further before cryopreservation and generation of Research Cell Banks (RCBs). To estimate productivity of the CC6.35 antibody-expressing stable CHO cell pools, both cell pools were expanded in a small-scale cell culture based on fed-batch process. After 14-days, the harvested supernatants were measured for titer by binding to a Protein-A biosensor on the Octet® System. Table 1 shows the percent (%) viability and expression levels (in g/L) for both EF1-CC6.35 and CMV-CC6.35 stable CHO cell pools.

Based on the higher titer, the CMV-CC6.35 cryopreserved RCB vials were advanced for use in scale-up production. This (CMV-CC6.35) RCB was tested for safety and regulatory acceptance for producing the two pivotal lots of material: one for preclinical safety studies and another for Phase 1 clinical trial. Prior entry of the RCBs in the cGMP facility, rapid safety test using PCR (Polymerase Chain Reaction) and NGS (Next-Generation Sequencing) based viral safety testing were performed to eliminate risk of adventitious contamination. In addition, the RCBs were also tested using compendial test for mycoplasma and sterility.

For producing material for preclinical safety study, the RCB vials were thawed and expanded using a seed-train to support a 200L bioreactor. The key cell culture process indicators included cell growth, cell viability, metabolic profiles (for ammonia and lactate), bioreactor regulation profiles (for pH, glucose, osmolality, and pCO_2), and antibody titer. After 14 days, the cell culture supernatant was harvested, clarified, and subjected to a 3-column chromatography platform purification process (involving Protein-A column chromatography, Anion-Exchange Column Chromatography, and a Cation-Exchange column chromatography) to generate purified Drug Substance (DS), formulated at 20 mg/ml in an antibody platform buffer as Drug Product (DP) for intravenous administration.

For producing material for the Phase 1 clinical study, a similar upstream (cell culture) process strategy was employed except that in this case, the RCB vials were thawed and expanded using a seed-train to support a 2000L bioreactor. After 14 days, the supernatant from the 2000L bioreactor was harvested, clarified, and purified via the same 3-column chromatography platform process to generate purified Drug Substance (DS), formulated at 20 mg/ml in a histidine-based buffer as Drug Product (DP) for intravenous administration.

Comparison of the key upstream process parameters between 200L and 2000L scale bioreactors are shown in Table 2. The two bioreactor runs were comparable in their overall metabolic profiles, except for the final productivity which was lower in the 2000L run. This was possibly due to an offset in general viability at end of the run

TABLE 1 Viability and expression (at Day 14) of non-clonal stable CHO cell pools expressing CC6.35 mAb in a 10 ml fed-batch culture

CHO cell pools	Viability (in %)	Expression level (in g/L)
EF1-CC6.35	99.44	3.05
CMV-CC6.35	99.20	6.01

TABLE 2 Comparison of upstream process parameters between 200L and 2000L scale bioreactors

Final bioreactor scale	Peak VCD (10^6 VC/ml)	Day 14 VCD (10^6 VC/ml)	Day 14 viability (%)	Day 14 titer (g/L)	Average qP (pg/cell/day)*
200L	24.90	19.00	87.40	1.89	8.34
2000L	21.40	14.40	81.80	1.32	6.25

Note: *Values calculated between Days 3 and 14.

Abbreviations: qP, cell specific productivity; VC, viable count; VCD, viable cell density.

TABLE 3 Comparison of parameters of the drug substances from 200L (non-cGMP) and 2000L (cGMP) production runs

Parameters	Specifications	DS (from 200L)	DS (from 2000L)
Appearance	Practically free from visible particles	Practically free from visible particles	Practically free from visible particles
Clarity and degree of opalescence (NTU)	NMT 18 NTU	5	3.4
Degree of coloration	Not more intensely colored than Reference Solution Y5	<Y6	<Y5
pH	5.0–6.0	5.6	5.5
Osmolality	250–400 mOsmol/kg	310	320
Protein concentration	18–22 mg/ml	20.6 mg/ml	19.8 mg/ml
Biological activity (ELISA)	70%–130% of Reference standard	95%	105%
Purity (reduced CGE-SDS)	NLT 85%	95.3%	96.2%
LMW (non-reduced CGE-SDS)	NMT 12%	8.3%	7.5%
% Purity by iCE	CPI cluster 3 (main peak): 97%–103% Acidic cluster: Report result Main peak: Report result Basic cluster: Report result	CPI% = 100 Acidic cluster: 51.0% Main peak: 39.1% Basic cluster: 9.9%	CPI% = 100 Acidic cluster: 49.4% Main peak: 40.5% Basic cluster: 10.1%
HMW species (SE-HPLC)	NMT 6.5%	4.3%	3.8%
Residual HCP	NMT 30 ng/mg	1.794 ng/mg	< 0.303 ng/mg
Residual DNA	NMT 5 pg/mg	<0.421 pg/mg	<0.22 pg/mg
Residual protein A	NMT 30 ng/mg	< 0.311 ng/mg	< 0.8 ng/mg
Bioburden	NMT 1 CFU/10 ml	0 CFU/10 ml	0 CFU/10 ml
Bacterial endotoxin test (BET)	NMT 0.1 EU/mg	<0.01 EU/mg	<0.01 EU/mg

Abbreviations: CFU, Colony Forming Unit; CGE-SDS, capillary gel electrophoresis-sodium dodecyl sulfate-polyacrylamide gel electrophoresis; CPI, cluster peak identification; ELISA, enzyme-linked immunosorbent assay; EU, endotoxin unit; HCP, host cell protein; HMW, high molecular weight; LMW, low molecular weight; NLT, not less than; NMT, not more than.

that contributed to an apparent decrease in the specific productivity. Despite the relative lower yield in the 2000L bioreactor, the amount of material produced was sufficient to cover the material requirements of the Phase I clinical study.

Both Drug Substances and Drug Products (200L and 2000L scales) were tested based on a proposed analytical test panel and results compared with agreed acceptance criteria. The analytical test panel, comprising of quantitative test (protein concentration and biological activity), qualitative tests (physico-chemical and microbiological purity) and compendial test methods, was set based on prior knowledge of antibodies of similar structure, and after confirmation of their suitability for the purpose. In addition, a formal analytical comparability was performed to ensure that the two products, from 200L and 2000L scales, are "essentially similar" despite the changes in their production scale and minor change in processes. The results of the analytical comparability study are reported in Table 3.

In addition to results in Table 3, Circular Dichroism (CD) analysis showed that both DS materials are comparable in terms of secondary and tertiary structures (data not shown). Thermal analysis (by Differential Scanning Fluorimetry) showed that both DS samples have similar thermal denaturation profiles with a temperature of onset (T_{onset}) at 60–61°C with two inflection points (IP) for both samples, one at 67°C and another at 78°C (data not shown). Extensive characterization of glycans (glycan mapping) of the two DS materials showed that the various detected N-linked glycan species are comparable, except for some minor differences in relative distribution of the galactosylated, fucosylated, and sialylated species (data not shown). Additionally, LC-MS analysis was performed on the two DS materials for intact mass and deglycosylated/reduced mass. Intact mass LC-MS analysis showed that the main proteoform for both non-cGMP DS batch and the cGMP DS materials was the intact molecule PyroQ-LC + HC[-K] coupled with FA2-FA2 glycans (data not shown). With respect to the expected mass of 150622.60 Dalton, the non-cGMP DS had an experimental mass of 150622.35 Dalton, whereas the cGMP DS had a mass of 150623.80 Dalton. Some other fragments were detected and identified in both samples, amongst which the most abundant was the LC-LC dimer (data not shown). After deglycosylation/reduction, the LC-MS analysis revealed that the two DS materials were overall comparable in terms of an intact molecule, except for slightly higher levels of HC C-terminal truncation in the cGMP batch (data not shown). Finally, the CC6.35 amino-acid

sequence predicted 16 total disulfide bridges and using non-reducing peptide mapping by LC-MS/MS, we found those disulfide bridges were detected and in line with expected (canonical) ones.

In summary, we demonstrate that using non-clonal stable CHO cell pools and use of platform processes can expediate early clinical development of monoclonal antibodies during pandemic outbreaks of emerging infectious diseases, such as COVID-19. We show that the antibodies produced from these stable CHO pools at two large scales bioreactors were comparable using platform upstream and downstream processes, and through robust analytical testing, they were deemed suitable for clinical use. Since clinical development timeline continues to thwart rapid evaluation of therapeutic and prophylactic interventions during pandemics and therefore improvements in development span essential (Kelley, 2020), we believe that this approach of using non-clonal stable CHO cell pools that enabled the manufacturing of early clinical trial material within 4.5 months, is a feasible alternative for rapid cGMP manufacturing and a means that can accelerate the pace of therapeutic and prophylactic protein evaluation in the clinic.

ACKNOWLEDGMENTS

We thank the various members of the Project Team at Merck KGaA and IAVI for their contributions. We thank Sowmya Rajendran, Lynn Webster, Surya Karunakaran and Ferenc Boldog at ATUM for Cell Line Development of the stable CHO cell line pools. We thank the members of the senior management team at Merck KGaA and IAVI for their support and guidance through the project.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

ORCID

Antu K. Dey https://orcid.org/0000-0001-7438-9379

REFERENCES

Bolisetty, P., Tremml, G., Xu, S., & Khetan, A. (2020). Enabling speed to clinic for monoclonal antibody programs using a pool of clones for IND-enabling toxicity studies. *mAbs*, *12*(1), 1763727.

Fan, L., Rizzi, G., Bierilo, K., Tian, J., Yee, J. C., Russell, R., & Das, T. K. (2017). Comparative study of therapeutic antibody candidates derived from mini-pool and clonal cell lines. *Biotechnology Progress*, *33*(6), 1456–1462.

Hu, Z., Hsu, W., Pynn, A., Ng, D., Quicho, D., Adem, Y., Kwong, Z., Mauger, B., Joly, J., Snedecor, B., Laird, M. W., Andersen, D. C., & Shen, A. (2017). A strategy to accelerate protein production from a pool of clones in Chinese hamster ovary cells for toxicology studies. *Biotechnology Progress*, *33*(6), 1449–1455.

Kelley, B. (2020). Developing therapeutic monoclonal antibodies at pandemic pace. *Nature Biotechnology*, *38*(5), 540–545.

Munro, T. P., Le, K., Le, H., Zhang, L., Stevens, J., Soice, N., Benchaar, S. A., Hong, R. W., & Goudar, C. T. (2017). Accelerating patient access to novel biologics using stable pool-derived product for non-clinical studies and single clone-derived product for clinical studies. *Biotechnology Progress*, *33*(6), 1476–1482.

Puck, T. T., Cieciura, S. J., & Robinson, A. (1958). Genetics of somatic mammalian cells. III. Long-term cultivation of euploid cells from human and animal subjects. *Journal of Experimetnal Medicine*, *108*(6), 945–956.

Rajendra, Y., Balasubramanian, S., McCracken, N. A., Norris, D. L., Lian, Z., Schmitt, M. G., Frye, C. C., & Barnard, G. C. (2017). Evaluation of piggyBac-mediated CHO pools to enable material generation to support GLP toxicology studies. *Biotechnology Progress*, *33*(6), 1436–1448.

Rajendra, Y., Balasubramanian, S., Peery, R. B., Swartling, J. R., McCracken, N. A., Norris, D. L., Frye, C. C., & Barnard, G. C. (2017). Bioreactor scale up and protein product quality characterization of piggyBac transposon derived CHO pools. *Biotechnology Progress*, *33*(2), 534–540.

Rajendran, S., Balasubramanian, S., Webster, L., Lee, M., Vavilala, D., Kulikov, N., Choi, J., Tang, C., Hunter, M., Wang, R., Kaur, H., Karunakaran, S., Sitaraman, V., Minshull, J., & Boldog, F. (2021). Accelerating and de-risking CMC development with transposon-derived manufacturing cell lines. *Biotechnology and Bioengineering*, *118*, 2301–2311.

Scarcelli, J. J., Shang, T. Q., Iskra, T., Allen, M. J., & Zhang, L. (2017). Strategic deployment of CHO expression platforms to deliver Pfizer's Monoclonal Antibody Portfolio. *Biotechnology Progress*, *33*(6), 1463–1467.

Stuible, M., van Lier, F., Croughan, M. S., & Durocher, Y. (2018). Beyond preclinical research: Production of CHO-derived biotherapeutics for toxicology and early-phase trials by transient gene expression or stable pools. *Current Opinion in Chemical Engineering*, *22*, 145–151.

Wilson, M. H., Coates, C. J., George, A. L. Jr. (2007). PiggyBac transposon-mediated gene transfer in human cells. *Molecular Therapy*, *15*(1), 139–145.

Wright, C., Alves, C., Kshirsagar, R., Pieracci, J., & Estes, S. (2017). Leveraging a CHO cell line toolkit to accelerate biotherapeutics into the clinic. *Biotechnology Progress*, *33*(6), 1468–1475.

Zhang, Z., Chen, J., Wang, J., Gao, Q., Ma, Z., Xu, S., Zhang, L., Cai, J., & Zhou, W. (2021). Reshaping cell line development and CMC strategy for fast responses to pandemic outbreak. *Biotechnology Progress*, *37*, e3186.

How to cite this article: Agostinetto, R., Rossi, M., Dawson, J., Lim, A., Simoneau, M. H., Boucher, C., Valldorf, B., Ross-Gillespie, A., Jardine, J. G., Sok, D., Burton, D. R., Hassell, T., Broly, H., Palinsky, W., Dupraz, P., Feinberg, M., & Dey, A. K. (2022). Rapid cGMP manufacturing of COVID-19 monoclonal antibody using stable CHO cell pools. *Biotechnology and Bioengineering*, 119, 663–666. https://doi.org/10.1002/bit.27995