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

Rita Agostinetto[1], Jessica Dawson[2], Angela Lim[2], Mirva Hejjaoui-simoneau[3], Cyril Boucher[3], Bernhard Valldorf[4], Adin Ross-gillespie[3], Joseph Jardine[5], Devin Sok[5], Dennis Burton[5], Thomas Hassell [6], Hervé Broly[7], Wolf Palinsky[3], Philippe Dupraz[3], Mark Feinberg[6], and Antu Dey[8]

[1]Merck Serono SpA
[2]EMD Serono Biotech Center Inc
[3]Ares Trading SA
[4]Merck KGaA
[5]The Scripps Research Institute
[6]International Aids Vaccine Initiative
[7]Merck Serono SA-Corsier-sur-Vevey
[8]Greenlight Biosciences Inc

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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 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-SARS-CoV2 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 pre-clinical 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 clinically-ready material within 4.5 months, in comparison to 12-14 months for production of clinical trial material via the conventional approach.

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*Rita Agostinetto[1],Jessica Dawson[2], Angela Lim[2], Mirva Hejjaoui 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[6,9,] **

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

[2]EMD Serono, Billerica, MA, 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, CA, USA

[6]IAVI, 125 Broad Street, New York, NY, USA

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

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

[9]Current address: GreenLight Biosciences Inc., 200 Boston Avenue, Suite 1000, Medford, MA, USA.

*Correspondence: Antu K. Dey; Email: adey@greenlightbio.com

KEYWORDS

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

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 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-SARS-CoV2 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 pre-clinical 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 clinically-ready material within 4.5 months, in comparison to 12-14 months for production of clinical trial material via the conventional approach.

COMMUNICATION TO THE EDITOR

Multiple cell line sources are used as hosts to produce recombinant prophylactic and therapeutic proteins for human use. Recombinant 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. To overcome this challenge, we wanted to evaluate the possibility of using non-clonal stable CHO cell pools to expediate production of a monoclonal antibody (mAb) against SARS-CoV2, CC6.35, particularly through cGMP manufacturing of a single batch of material for a Phase 1 clinical trial. Since this approach 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 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). In order 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 supernatant was 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.

For producing material for pre-clinical safety study, the RCB vials were thawed and expanded using a seed-train to support a 200 L 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) steps to generate purified Drug Substance (DS), formulated at 20 mg/mL in an antibody platform buffer as Drug Product (DP) for intra-venous 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 2000 L bioreactor. After 14 days, the supernatant from the 2000 L 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 intra-venous (i.v.) administration.

Both Drug Substances and Drug Products (200L and 2000 L scales) were tested based on a proposed analytical panel and results compared to agreed acceptance criteria. The analytical panel, comprising of quantitative test (protein concentration and biological activity), qualitative tests (physico-chemical and microbiological purity) and compendial methods, was set based on prior knowledge on 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 2** .

In addition to results in **Table 2** , 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 intact molecule, except for slightly higher levels of HC C-terminal truncation in the cGMP batch (data not shown). Finally, non-reducing peptide mapping by LC-MS/MS showed that the (nine) detected disulphide bridges were aligned 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 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 tested they were deemed suitable for clinical use. Since clinical development timeline continue to thwart rapid evaluation of therapeutic and prophylactic interventions during pandemics and improvements in development span essential (Kelley, 2020), we believe that this approach of using non-clonal stable CHO cell pools, which enabled 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 clinic.

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TABLES

Table 1. Viability and expression (at day 14) of non-clonal stable CHO cell pools expressing CC6.35 mAb in a 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 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

Parameters	Specifications	DS (from 200L)	DS (from 2000L)
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	18-22mg/mL	20.6 mg/mL	19.8 mg/mL
Biological activity (ELISA)	70-130 % of Ref. STD	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/10mL	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

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