WHITE PAPER

Leveraging efficiency from cell line development to clinical manufacturing of monoclonal antibodies

Increase speed, reduce risk and maintain quality by partnering with a CDMO that has a comprehensive program for moving from discovery to clinical batches of a new biologic.

Given the relative value of being first to market, biopharmaceutical companies are looking to shrink the 10 or more years it usually takes for a biologic candidate to progress from drug discovery to regulatory approval.[2]

Following rapid antibody discovery, Curia continues to leverage proprietary technology, creative process engineering using single-use equipment and meticulous quality control for the most efficient route to GMP manufacturing of clinical phase drug substance in as little as one year (Figure 1).

Figure 1. From cell line development to Phase I drug substance in 12–16 months*

Stages from cell line development to clinical manufacturing and batch release. Once a promising lead has been discovered, it progresses to cell line development, process development and manufacturing.

**Timelines are subject to change depending on the manufacturability of the candidates.*

The process begins with rapid antibody discovery

Getting new mAbs to market quickly requires a combination of efficiency and expertise beginning in the discovery phase. The objective during discovery is to explore a wide range of sequences against a specific antigen target and then winnow down to find one to be tested in clinical trials.

At Curia, our First-to-Human discovery service optimizes this progression from hits to leads in as few as 90 days. Our tried and tested workflow is detailed in our white paper, Antibody-based drug discovery at the speed of light, and includes these steps:

- Immunize PentaMice® wildtype mice and/or human antibody-producing transgenic mice
- Isolate single B cells and screen as many as 80,000 cells using the Berkeley Lights® Beacon® Optofluidic system
- Complete high-throughput processing, enabled by automated screening coupled with unique barcoding to multiplex hits
- Sequence the variable heavy- and light-chain sequences of the mAb using NGS
- Analyze the diversity of sequences and assess antibody developability
- Reformat and express high-priority mAbs using our TunaCHO℠ transient antibody production process to yield milligram quantities for lead selection assays
- Perform kinetic analysis and epitope binning by surface plasmon resonance using the Carterra® LSA® platform

Curia's lead generation platform rarely requires further optimization, such as affinity maturation, thus saving additional development time. The most promising unique sequence is then handed off for cell line development and manufacturing to speed the mAb through to IND-enabling studies.

Cell line development using CHO-GSN℠ platform

PROPRIETARY STABLE EXPRESSION VECTORS

The mAb heavy- and light-chain gene sequences are inserted into a high-expression plasmid. These bicistronic vectors have a non-CMV promoter to avoid potential epigenetic promoter silencing and are specific for robust antibody expression. Curia also has monocistronic plasmids for proteins and complex molecules with three or more chains.

CLONAL SELECTION IN CHO-GSN CELLS

The plasmid is used to transfect Curia's proprietary and royalty-free CHO-GSN cell line, which is designed for high-level expression of target sequences and is genetically stable for at least 80

generations, exceeding the time the cells are used in manufacturing. It is derived from a CHO-K1 glutamine synthetase (GS) knockout and has strong GS selection and high titers.

Characterization and testing

It can take 4–6 weeks to generate a stable pool of cells, after which the cell bank is tested for sterility, mycoplasma and gene copy number. The latter is important because ectopic integration of the vector into the genome of CHO-GSN cells is random and can occur multiple times for the same cell. Clonal selection from this heterogeneous pool will result in a unique cell line with one integration, which is ideal for GMP manufacturing. A master cell bank containing the stable cell line will be used to make large amounts of mAb for clinical manufacturing.

Cell bank generation and characterization

A subcloning step confirms the cell line was indeed derived from a single cell. This traditionally involves limiting dilution, which is a statistical method in which cells are diluted to the point that, on average, every third well on a plate receives a single cell.

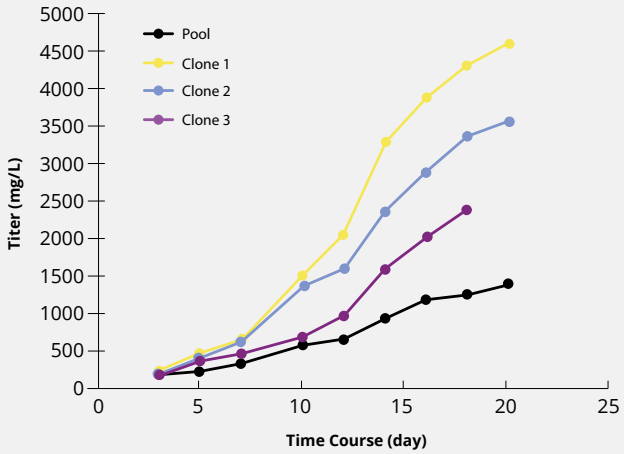
To prove the line is clonal, individual cells are placed in wells using an imager to provide documentation to support application for an IND. Limiting dilution is onerous and time-consuming, opening the door for more efficient alternatives.

SINGLE CELL SEEDING WITH SOLENTIM® VIPS™ IS FASTER

Making a cell bank is more efficient using Solentim VIPS (verified *in situ* plate seeding) for single cell cloning. A suspension of cells flows through a narrow stream of solution and the optics capability of VIPS provides proof of image-verified monoclonality. This avoids the subcloning step necessary during the limiting dilution method and effectively halves the single cell cloning timeline.

Monoclonality is confirmed with Solentim® Cell Metric®. In conjunction with upstream process development, a 50-mL production run assesses titer, which can be as high as 5 g/L (Figure 2).

Figure 2. mAb titer profile



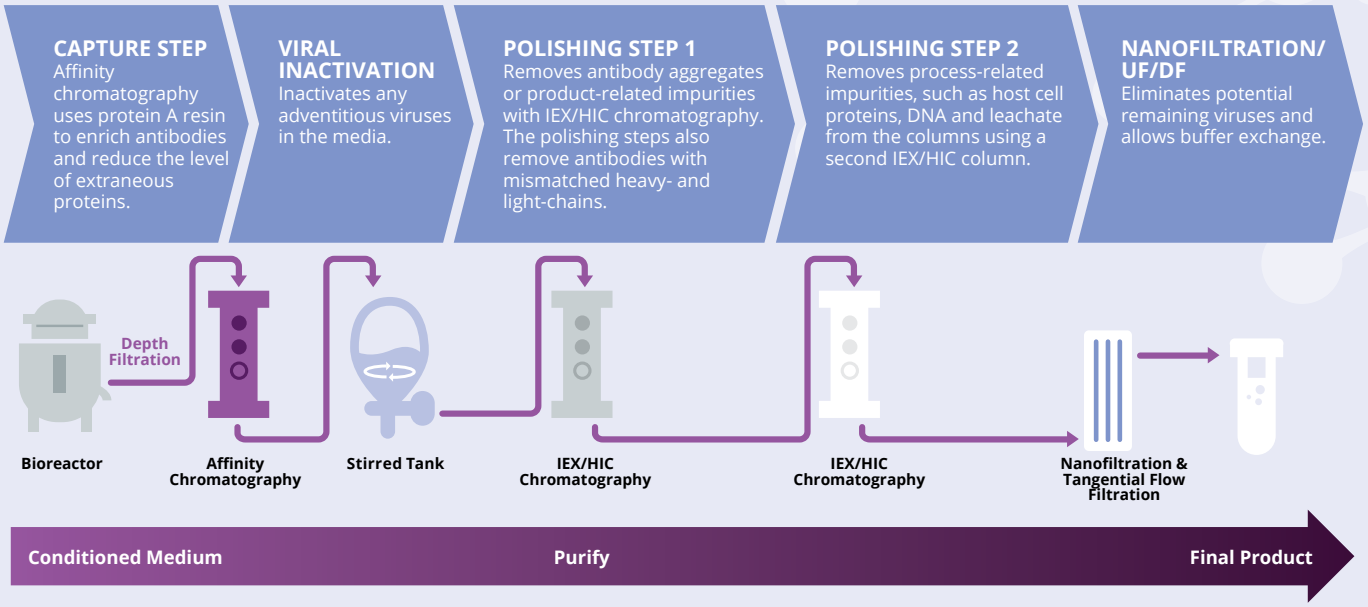
Upstream process development

The goal of upstream and downstream process development is to optimize growth of the cell bank, maximize antibody expression, optimize purification and promote consistency of each production run. The characteristics of the mAb should remain unchanged throughout the entire process, all the way to the GMP manufacturing batches that will be used for Phase I clinical studies.

OPTIMIZED CULTURE CONDITIONS WITH THE SARTORIUS® AMBR® 15 CELL CULTURE BIOREACTOR SYSTEM

Curia evaluates key parameters using the Sartorius Ambr 15 Cell Culture Bioreactor System, a high-throughput bioreactor capable of parallel-testing as many as 48, 15-mL cell cultures. Only proprietary animal-derived component-free (ADCF) media formulations are used. Testing antibody production under various test conditions (e.g., media) at such a small scale greatly improves the efficiency and cost effectiveness of process optimization.

Figure 3. Downstream process development



IEX/HIC = ion exchange/hydrophobic interaction chromatography. UF/DF = ultrafiltration/diafiltration.

The selected culture parameters are then used to test cell line scalability in multiple 2-L single-use bioreactors (SUBs). The next step is scaling up to a 50-L SUB for a confirmation run, then progression to 200–2,000-L SUBs. Curia is capable of delivering kilogram quantities of clinical phase material.

Downstream process development

Downstream process development is undertaken concurrently with upstream process optimization. This develops a multistep mAb purification process with the goal of minimizing process impurities, such as host cell protein, host cell DNA and extractables from chromatography resins. A representative downstream process is shown in Figure 3.

CHOICE OF RESINS IS IMPORTANT

Based on decades of experience, Curia has preferred resins suitable for GMP use for each of the purification steps. Each resin is procured from approved suppliers and qualified to ensure that it conforms to specific requirements.

Given the global supply chain bottlenecks exacerbated by the COVID-19 pandemic, it is critical to maintain sufficient inventories to ensure ample supply so that manufacturing schedules are not disrupted.

BATCH RECORD CREATION

GMP drug substance production readiness follows a detailed project plan, including assessment of facility, equipment, bill of materials, material specifications, solution preparations, sampling plan, master batch record creation and resource scheduling. Facility and equipment fit are evaluated at the initial project proposal phase. Curia maintains on-hand inventory of common materials for the mAbs platform. A risk assessment is performed on new critical raw materials to help establish material specifications.

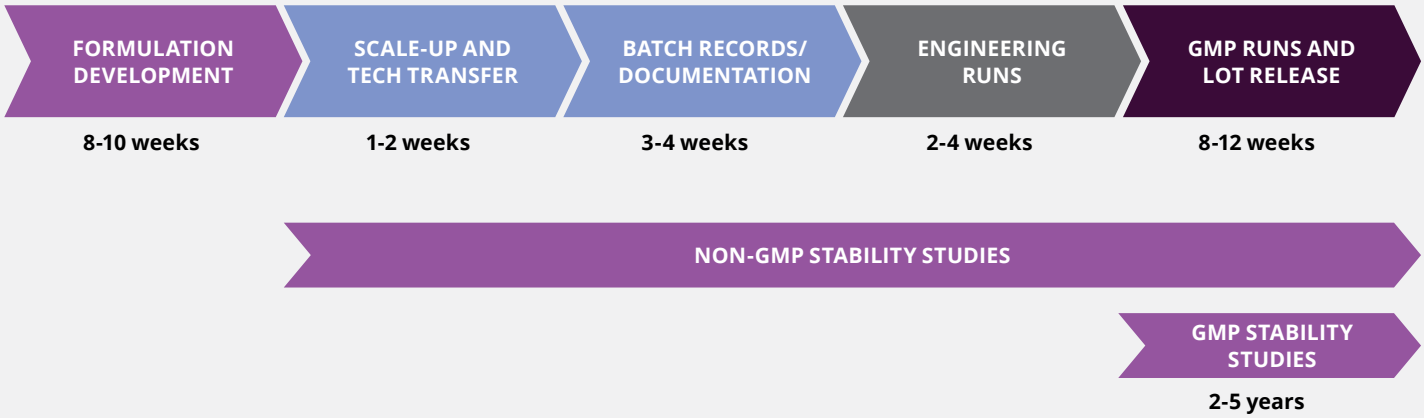
Curia has GMP capability for media, buffers and ancillary solution preparations with QC testing. Master batch records are created using templates populated with information from PD technical transfer documentation containing formulations, process parameters and in-process controls.

Restricted access cleanrooms undergo routine environmental monitoring in classified areas. Proper changeover and line clearance prevent cross contamination and mix-ups.

Drug product formulation development and manufacturing

Curia has multiple facilities capable of performing formulation and fill-finish, covering small molecules, injectable drug products, mAbs and other biologics and cytotoxics. The path from drug product formulation to fill-finish is dealt with in greater detail in our white paper, The benefits of end-to-end formulation and fill-finish of biologics. The stages and timeline are outlined in Figure 4.

Figure 4. Stages and timeline for drug product development



The timeline outlined here is a typical scenario for a well-behaved product. Curia takes a bespoke approach to each project and applies appropriate timelines based on the needs of each program.

Identifying critical quality attributes (CQAs)

The first phase of formulation development is a preliminary characterization of the protein to identify both its physical and chemical CQAs, which could result in product-related and process-related impurities. Formulation development is based on the most relevant ICH stability guidelines, which promote the ultimate goal of drug product stability. This approach uses common stresses encountered during pharmaceutical processing, shipping and storage:

- Storage temperature
- Agitation and shear stress
- Freeze/thaw cycles
- Photosensitivity to UV and visible light
- Liquid stability of lyophilized formulations

Observing the behavior of a protein across a wide range of conditions (e.g., pH, ionic strength, excipients, surfactants) under a variety of stresses allows for the identification of potential degradation products. This also verifies the stability-indicating nature of analytical assays.

FORMULATION OPTIMIZATION

Knowing the CQAs and critical stresses of the mAb dictates the direction of the rest of development

and may lead to a formulation optimization strategy. Here, we look at additives to the formulation that address any problems and, ultimately, achieve stability over time. This data-driven process asks what, if anything, needs to be added to the formulation to help prevent relevant degradations that have previously been identified, and that the addition of these additional excipients doesn't create other issues.

CREATING THE MOST COMPETITIVE PRESENTATIONS

The method of administration chosen can affect the profitability of a drug product. Many patients prefer the convenience of an injectable device they can use at home, instead of having to go to a clinic to have their medicine administered with an IV. In these cases, the preferred competitive presentation will be a subcutaneous, pre-filled syringe or autoinjector. More than half our customers come to us already having an acceptable IV formulation, but are looking to increase the concentration of mAb to create a subcutaneous formulation.

While creating the ideal formulation is data driven and can sometimes be straightforward, it also relies on time-tested experience, knowledge of complicated protein dynamics and current best practices of formulation and GMP manufacturing. The success of formulation development depends on keeping in mind the end goal of a process that can be scaled up to GMP fill-finish, withstand process-related stresses and maintain stability for the intended duration. Formulation scientists are often challenged with aggressive target product profiles and need to always maintain a methodical yet creative approach to solving these challenges.

FILL-FINISH

Curia's R&D division has two clinical drug product sites, each offering fill-finish using single-use equipment, which eliminates the need for cleaning protocols and validation. Each site has automated fill-finish lines with combined technologies covering restricted access barrier systems (RABS), liquid/lyophilized/suspension capabilities and vial/syringe/cartridge formats.

THE BENEFIT OF INTERNAL SCALE-UP

Once a formulation has been developed in the R&D lab, the process is scaled up into clinical manufacturing. The clinical manufacturing team has to generate batch records to capture all the steps succinctly. It requires changing many aspects of the process, like altering dialysis to UF/DF, and converting from volumetric to weight-based formulation. Having a formulation R&D team familiar with these limitations in GMP manufacturing gives an advantage during initial formulation work.

When this is an internal tech transfer, the R&D team can work directly with the formulation scientists to develop a more efficient process to alleviate risk and save time and money. Internal collaborations lead to successful engineering runs, the goal of which is to gauge the acceptance criteria and make sure everything will behave appropriately during a subsequent GMP batch.

Quality systems at Curia

Curia's quality teams function independently of the development and manufacturing teams and follow US and EU regulations and guidelines, as applicable to the products manufactured. Curia's Hopkinton, MA manufacturing site also has ISO 13485:2016 certification, which signals our commitment to international standards of quality.

Comprehensive platforms for development, manufacturing and analytics of biologics enhance our quality systems. Control of cross-contamination is maintained through single-use manufacturing processes, as well as fully independent suites for different types of manufacturing activities (e.g., mRNA and mAb production). Our single-use systems provide the flexibility to allow efficient production and faster development time.

ASSAYS AND ANALYTICAL SERVICES

Curia's analytical development team develops the assays needed for QC to test mAbs to ensure a product meets specifications. This is a standard set of assays for a typical antibody program to ensure the purity, consistency and activity of the antibody (Figure 5). These are performed on every batch of drug product, for both engineering runs and GMP manufacturing runs.

Figure 5. Antibody assays and analytics

Test	Purpose	Assay
Appearance	Safety, quality	Visual Inspection
Bacterial Endotoxin	Safety, quality	Chromogenic LAL
Sterility (w/BnF)	Safety, quality	1 mL x 2 Direct Inoculation
pH	Safety, quality	pH
Osmolality	Safety, quality	Osmolality
RNA Concentration	Strength	UV A260
RNA Identity	Identity	CE-Based
Identity (as RNA)	Identity	Enzyme Degradation and CE
Identity (RNA sequence)	Identity	NGS
Residual Protein	Purity	BCA, PAGE, Fluorescent
Residual DNA	Purity	qPCR
Residual DS RNA	Purity	Dot Blot, ELISA
Residual DNA:RNA Hybrids	Purity	Dot Blot, ELISA
RNA Integrity	Quality	CE, HPLC
% Cap	Strength	LC-MS, CE
Functional Assay	Strength	Optional

FOCUSED ON MULTIPLE REGULATIONS

When working with a customer for clinical-phase drug substance manufacturing, it is important to know the relevant set of US and EU quality regulations and guidelines. In the US, a series of cGMP regulations ensure the safety and quality of drug substances, drug products and medical devices, such as:

- **21 CFR Part 820**
  This is needed if a mAb is used in a diagnostic application, since diagnostics fall under medical devices and are covered by Part 820.

- **21 CFR Parts 210 and 211**
  Drug substances, such as mRNA, are included in these regulations that lay out cGMP for manufacturing, processing, packing and holding of pharmaceuticals.
- **21 CFR Parts 600 and 610**
  These are the general product standards for biologics.

In the EU, GMP drug production is governed by:

- Commission Directive 2003/94/EC, laying down the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use.
- Volume 4 of "The rules governing medicinal products in the European Union," which contains guidance for the interpretation of the principles and guidelines of good manufacturing practices for medicinal products for human and veterinary use laid down in Commission Directives 91/356/EEC, as amended by Directive 2003/94/EC, and 91/412/EEC respectively.

Curia quality teams work with customers to ensure that the appropriate standards are planned and executed.

QUALITY AGREEMENTS

These documents define the duties for the quality teams at both the CMO and the customer for all contract GMP activities. This applies to:

- Bulk drug substance for clinical use (mammalian, mRNA)
- cGMP testing services
- Cell banking under GMP
- Drug product for clinical use

Curia quality teams work with customers to ensure that the appropriate standards are planned and executed.

Although some specific requirements for drug substance and drug product production need to stay in Curia's templates, some clients may prefer to use their internal templates to outline quality responsibilities. In these cases, Curia makes sure the quality agreements are aligned between the CMO and the customer.

AUDIT HISTORY

- Regular customer audits
- Annual ISO surveillance audits (Hopkinton site)
- Multiple qualified person (QP) assessments
- California State inspections (license in place at Camarillo, CA site)

Specializing in First-to-Human manufacturing support

Curia's advanced technology and capabilities span all phases of drug manufacturing, from antibody discovery and engineering services to manufacturing that supports clinical development. We offer complete development services or accept tech transfers.

Want to learn how our efficient tactics can progress your mAb from discovery to clinical manufacturing in as little as 18 months? Visit curiaglobal.com/biologics.

ABOUT CURIA

Curia is a global contract research, development, and manufacturing organization (CDMO) with over 30 years of experience. With an integrated network of 20+ facilities worldwide and a team of 3,000+ dedicated professionals, we specialize in partnering with biopharmaceutical customers to bring life changing therapies to market. Our offerings in small molecules, generic APIs, and biologics span discovery through commercialization, with integrated regulatory, analytical, and sterile fill-finish capabilities. Our scientific and process experts, along with our regulatory-compliant facilities, deliver a best-in-class experience across drug substance and drug product manufacturing. From curiosity to cure, we are your trusted ally in accelerating life-changing therapeutics. ***Learn more at curiaglobal.com***

Reference

1 Matthews H, Hanison J, Nirmalan N. "Omics"-Informed Drug and Biomarker Discovery: Opportunities, Challenges and Future Perspectives. *Proteomes* 2016;4(3):28. https://www.mdpi.com/2227-7382/4/3/28#cite

CONTACT US

www.curiaglobal.com

curia