Simplifying Progress

A Guide to Accelerating Cell Line Development for Commercial Production

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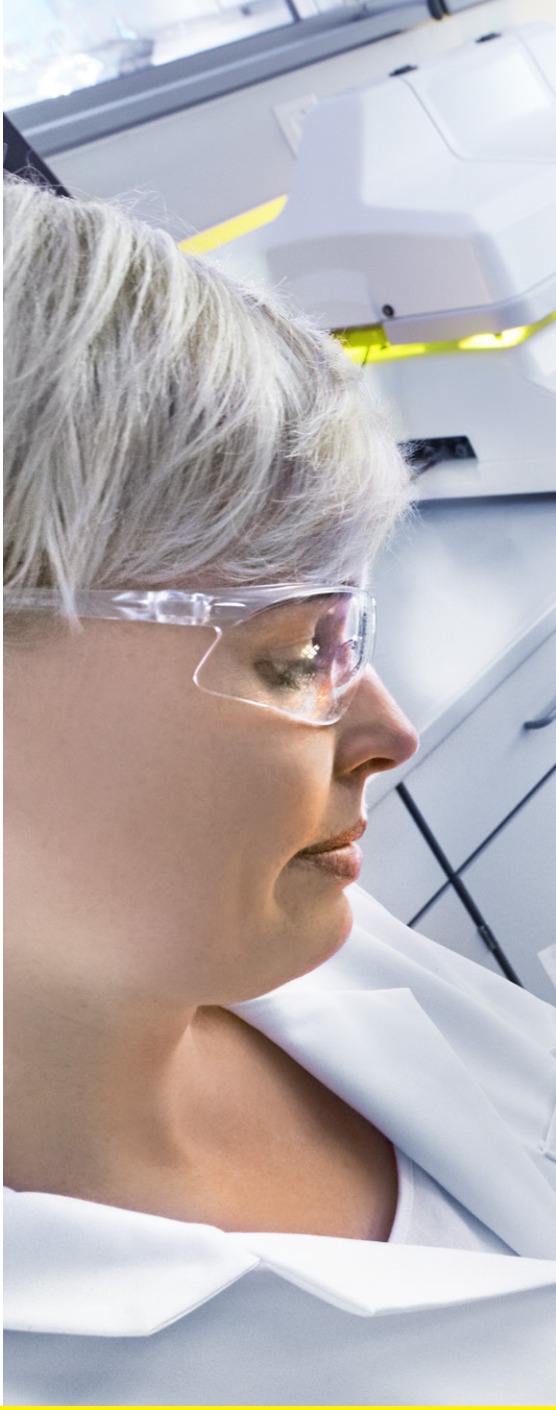
Cell Line Development: The Foundation of a Reliable Production Process



Biopharmaceutical drug products, or biologics, are fast becoming an essential branch of the healthcare industry. Primarily consisting of therapeutic recombinant proteins, biopharmaceuticals have had considerable success in treating various diseases, particularly cancers and inflammatory disorders (1). However, developing a reliable process for commercial biopharmaceutical production is logistically and technically demanding, with many opportunities for projects to break down.

Biopharmaceutical manufacturing employs living host cells as production centers. The development of a robust, high-yielding cell line is the foundation of a reliable production process. However, cells introduce a source of variability and a unique set of technical challenges.

Early awareness of the possible obstacles to efficient process development is critical for making informed project decisions. Here, we discuss the challenges associated with cell line development in the production of biologics before outlining solutions that can help biotech developers evade the pitfalls of misinformed decision-making.

Process of Cell Line Development

Creating a robust, stable cell line that can yield sufficient material for testing, optimization, and manufacturing at commercial scales is a crucial step in bioprocess development. The route taken during the early stages of your project will determine its future success or failure. The following outline presents an overview of the cell line development workflow.

1. Gene cloning and transfection
Firstly, cells of the selected mammalian host cell line must be engineered to express the desired protein.
An expression vector containing the genetic material required to encode the therapeutic protein must be synthesized and used to transfect the cells. The transfection process creates a mixed population of cells, which are then subject to selection, pooled, and analyzed. Early analysis provides insights into the general characteristics of the cells carrying the recombinant DNA and their ability to produce the anticipated biologic.

2. Clone screening and selection
Next, single cells must be isolated and expanded to create clonal populations. Screening is subsequently performed to identify the most desirable clones. High-performing clones are stable and capable of producing the desired protein at high titers. The monoclonality of the cell population should also be verified before proceeding to further characterization.

3. Cell line characterization
Once screening and clone selection is complete, the best performing clones should be expanded and fully characterized to ensure the cells can recapitulate all the features of the desired biologic. Multiple assays should be employed to evaluate critical quality attributes (CQAs) of the product, such as potency, binding, and physicochemical and structural properties. Additionally, next-generation sequencing should be used to examine the transgene insertion site and provide further insights into the clone's stability.

4. Expansion and optimization
Achieving the optimal production parameters at different scales can require several rounds of optimization. Developers must verify that product quality is maintained from pilot scale through to commercial scales. Iterative testing of various media compositions and process parameters can identify further opportunities to improve the efficiency and yield of protein production at the necessary manufacturing scales.

5. Cell banking
Finally, the cells are expanded to create high-density cell banks for the continuous production of the biopharmaceutical. GMP-compliant master cell banks are established, fully tested, and characterized in preparation for creating working cell banks and the beginning of manufacturing.

Cell Line Development Key Challenges

There are various stages at which problems can arise during the creation and optimization of robust cell lines. However, a heightened awareness of potential future challenges during early process development can help mitigate the risk of failure, reduce the expenses associated with repeating processes, and ensure that the cell line and biotherapeutic meet regulatory requirements.

Choosing the Right Cell Line

Biologics are typically large and highly structured molecules. The mechanisms behind their expression, processing, packaging, and transport inside the cell are complex. As a result, it can be technically challenging to generate a cell line that reliably synthesizes the product and accurately captures its features, which may be necessary for its clinical function.

The decision of which cell line to use for biopharmaceutical production is crucial, as its performance underpins the efficiency of the entire production process. A poorly functioning cell line can require developers to veer from established protocols and incur costs associated with performing multiple rounds of optimization.

A suitable cell line will be easy to transfect | transduce and produce the desired biologic with high efficiency.

The selected cell line must have the capacity for creating all biological features of the product, including structural moieties and post-translational modifications. Additional concerns include the cell line's stability, history, growth characteristics, and required culture conditions.

Chinese hamster ovary (CHO) cell lines are the most common choice for the manufacture of recombinant proteins, owing to their established track record of efficient protein production, among other factors (2). However, various other options, including human cell lines, might prove more suitable for some applications, such as avoiding potentially immunogenic non-human post-translational modifications (3).

Clone Selection

Screening cells to identify and select high-performing clones is perceived as a complex and arduous process. Clone performance can be unpredictable, so the higher the number of clones screened, the greater the chance of discovering a rare, very productive cell line.

Effective screening strategies can require several rounds of optimization and cause significant delays to process development. Once a few efficient clones have been identified, multiple assays should be carried out to build a thorough picture of the features of each before selecting the best performing clone. Performing these assays could require a range of expertise and can be labor- and time-intensive.

Clone selection should also be performed under representative conditions, ensuring decisions made about which clone to take forward will be applicable under a controlled bioreactor environment. For example, shake flasks can select clones that perform better under low dissolved oxygen or poor pH control, conditions unlikely to be present in bioreactor cultures.

Early Characterization and Optimization

Once screening and clone selection is complete, the selected clone (or a few clones) must be expanded and fully characterized. The biologics produced should be subject to comprehensive analysis using orthogonal methods to demonstrate safety and efficacy.
In addition to standard safety and functional assays, biosimilar products must undergo further testing to establish comparability to the innovator product and confirm the absence of any clinically relevant differences.
A related concern is ensuring the consistency of the manufacturing process is maintained; any fluctuations in cell growth conditions can significantly affect the quality of the product.

Comprehensive characterization requires the use of diverse assays, each providing different information about the product's properties and biological function. These analyses are likely to involve the use of multiple instruments, platforms, and methodologies. Additionally, evaluating the features and quality of the product requires analysis and integration of datasets from different technologies. This demands significant expertise across multiple disciplines, which might be unattainable for small biotechs. Finally, repeated sampling also creates opportunities for errors, inconsistencies, and contamination of the cell cultures and products.

Scalability

One of the most significant obstacles during the production of biologics is in scaling up procedures to accomplish commercial manufacturing. Making decisions using models that might not represent the conditions at larger scales can be a barrier to successful commercialization.

Additionally, scaling up usually involves transferring the process to intermediate scales and substantial optimization at each, which is costly and can represent a source of significant delay.

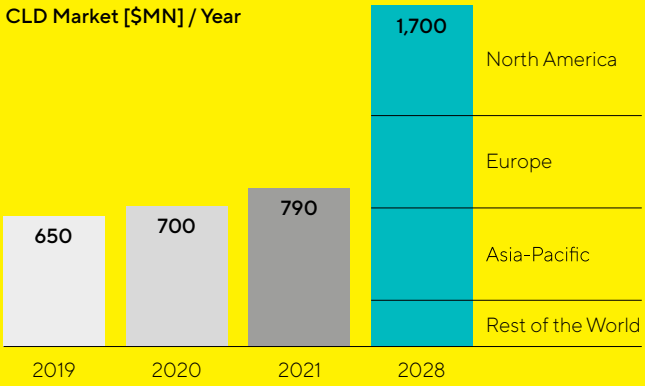
Logistics

As well as the technical challenges, cell line development exhibits several logistical complexities. An important consideration that must be made early during process development is whether the organization has the capacity to establish and test new cell lines, and if so, what technology platforms they will employ.

Developers must assess whether it is efficient and economical to carry out the entire process from start to finish in-house; outsourcing some steps may more productive and cost-effective.
Cell line development and maintenance require GMP-compliant facilities and a dedicated team with the necessary skill set to manage and monitor operations according to the required regulatory standards.

Cell Line Development Facts & Figures

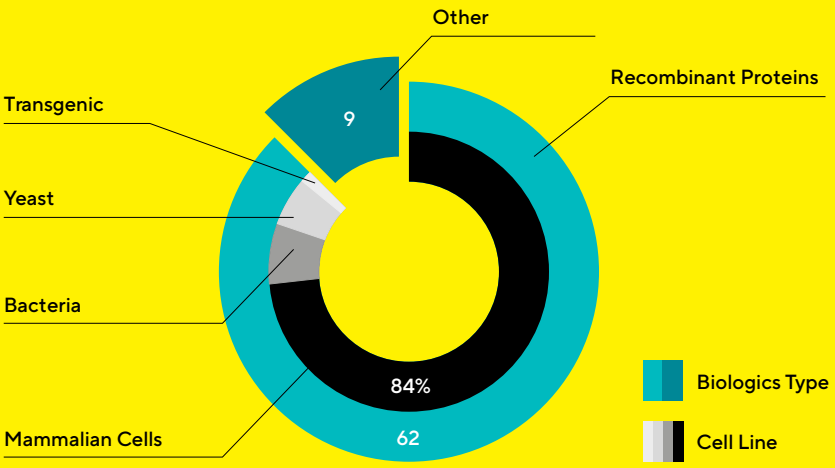
The global cell line development service market is expected to reach **$1.7 billion** in revenue by **2028**.

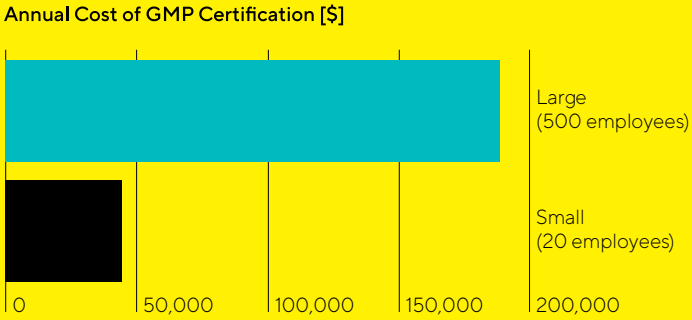


North America is expected to command over a **third** of the **market share**.

Cell line development is a **time-consuming** process – GMP grade cell lines are typically developed in **12-18 months**.

62 of the 71 newly approved biopharmaceutical active ingredients in the last 4 years were **recombinant proteins** and of those, **84%** were from **mammalian cells**.





GMP certification can be **pricey** – for small biotechs (<20 employees), the FDA estimates initial costs of around **$26,000, plus $46,000 annually**.

Overcoming The Obstacles

There are various solutions that small- and medium-sized biotechs can explore to reduce the risks associated with biologics development. Potential actions could include outsourcing some aspects of the project to external organizations with the necessary expertise to meet developer needs quickly. Other solutions could be implemented in-house and involve investing in some of the most advanced technologies and characterization services in commercial biologics production.

Established Cell Lines

Using an established expression platform can significantly reduce your project timelines and limit some of the uncertainties associated with biopharmaceutical development. An example of such a platform is the Cellca Cell Line Development system, a scalable, versatile biologic production platform with its own fully defined media system offered by Sartorius. Specialized cell lines limit the need for iterative optimization of a new cell line; they are designed to reduce variability and maximize yields and efficiency and significantly reduce development timelines. For example, the Cellca Cell Line Development technology allows developers to progress from DNA to research cell banks in as little as 14 weeks and facilitates direct scalability from small scale to large scale production.

Additionally, established cell lines will have a full history and a demonstrated track record of successful projects, giving developers confidence in their cell line development project.

Users can attain the Cellca CLD Technology License to produce their biologic in-house. Efficiently developing a reliable cell line requires a robust infrastructure and significant expertise. However, the Cellca CLD Technology is a plug-and-play system designed to simplify cell line development, with comprehensive support provided throughout the project.

Established Vectors

As well as employing an established cell line to streamline downstream process development, verified expression vectors can also be taken advantage of when developing a cell line in-house. Cellca Expression vector systems are flexible tools carefully designed to facilitate enhanced, stable expression of the inserted genetic material while minimizing any secondary effects on the host cells.

Early investment in a cell line development service eliminates the need for a trial-and-error approach and could be more economical in the long run. Purpose-built cell lines can be accessed by outsourcing some or all of the development process to an external company such as Sartorius. These services are run by expert scientists with experience in dealing with expression platforms for large, complex molecules, which are often challenging to express.

External Cell Line Development

Developers should carefully consider their capacity for commercial-scale, GMP-compliant production before beginning cell line development. In some cases, outsourcing the most time-consuming or operationally challenging parts of the project can be the best option, giving developers access to the latest technology and expertise without the associated logistical and regulatory challenges.

However, biotechs will need to carefully design their platform to facilitate smooth transfer between facilities during later stages.

Outsourced cell line development has grown in popularity (4), and developers now have a considerable choice when selecting the service that best matches their needs. A suitable service partner will have an established track record at delivering successful cell lines for protein production and will provide access to significant expertise in cell line generation.

The Cellca Cell Line Development platform from Sartorius provides services from initial clone selection to research cell bank, meaning you do not have to invest in the facilities, reagents, and labor hours to ensure you select and expand the best cell line in the optimal conditions for your production process. Such comprehensive solutions allow you to streamline the cell development process and reduce your time to market.

Cell Line Characterization Packages

Cell line characterization should be carried out throughout the drug development process to ensure biosafety regulations are met and that the product is behaving as expected. To avoid the expense of installing and acquiring proficiency in multiple technologies, many biotech companies often employ external service providers

to carry out all the necessary analysis to ensure their cell banks meet regulatory requirements and can be released. Sartorius offers pre-determined cell line characterization packages designed to comprehensively analyze your cell line in accordance with ICH regulatory guidelines. Ready-to-use packages include a range of assays and can be customized to fit the needs of any project.

High Throughput | Automated Technologies

If economically viable, accessing the latest analytical platforms and bioprocessing technologies could support large screening projects.

High-throughput, automated solutions for cell line development and expansion, such as the Ambr® multi parallel bioreactors, can save significant man-hours, optimization time, and resources (5). Such systems facilitate screening under physiologically relevant conditions within a scalable platform earlier on in the development process, incorporating process development and quality by design (QbD) principles, shortening timelines, and reducing risk at later stages.

The Ambr®15 provides reproducible conditions for up to 48 parallel cultures in a single run, and the integrated analyzers allow users to seamlessly track and record their cultures to facilitate efficient optimization. Automated platforms also enhance the scalability of the production process; conditions are consistent between scales, allowing you to make more informed predictions about your strategy as you scale up to commercial manufacturing (6).

Consolidated platforms reduce the need to install and master several technologies. An integrated suite of technologies work smoothly together, supporting progress from the beginning to the end of the project. Integrated analytics, such as automated cellular and metabolite analysis, and data analysis, such as the Umetrics® Suite for DoE and Multivariate Data Analysis (MVDA) data analytics software, provides further contro over processing and analysis, empowering users with an abundance of high-quality data in a user-friendly interface. These features enable greater process understanding and guide informed decision-making.

Traditional methods of analysis, such as ELISA, are useful in assessing product titer but generate limited information on each clone's critical productivity attributes. Advanced technologies, like the iQue® Advanced Flow Cytometry Platform, provide deeper insights to facilitate the ranking and selection of production clones with rapid and simultaneous evaluation of titer, productivity, and cell viability (7). Clone ranking can change dramatically when all these attributes are taken into account (8).

While expression level analysis like titer screening is carried out early in cell line development, other CQAs, such as glycan characterization, are often only assessed later in the development process due to a lack of appropriate and high-throughput analytical techniques that can be used to perform rapid screens.

The use of advanced, label-free detection systems accelerates comprehensive molecule analysis. For example, the Octet platform uses Bio-Layer Interferometry (BLI) to detect real-time binding of molecules as a means of quantification or for kinetic analysis. The ability to measure protein titer and glycosylation in crude cell culture supernatant in real-time brings confidence in clone selection and streamlines the workflow.

Ultimately, integrated, automated technologies simplify and accelerate your cell line development towards process optimization and reduce variability in your production process.

Robust Cell Line Development Requires Well-Informed Decision-Making

The challenges of biopharmaceutical development mean projects are often associated with significant risk. Potential hurdles include technical challenges due to the complex nature of biologics and logistical issues as a result of material requirements.

Developers want to establish robust and reliable pipelines that reduce their time to market while keeping costs low. However, the timelines and expenses budgeted are often idealistic. To speed up process optimization, they require rapid access to high-quality data to inform decision-making.

Making process decisions with the limited information available at the early stages is challenging. However, a robust design, as well as time and capital investments, can maximize efficiency in the long run. Effective risk-management strategies include early process and product characterization, utilizing external expertise, and taking advantage of purpose-built technology and services.

Timely adoption of automated and high-throughput platforms from the beginning of a biotherapeutics project development can streamline optimization strategies in a traceable way. This can significantly improve cell line development efficiency by facilitating the assessment of multiple parameters in parallel, with conditions maintained from research scale to commercial-scale production.

Author Bio

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Fern Slingsby has been working for Sartorius since 2018. She obtained her undergraduate degree in Molecular Biology from Queen Mary, University of London, and her PhD from the Medical Research Council (UK) | University of Edinburgh.

Fern has over 18 years of experience in recombinant protein production and upstream development. Her background includes six years as a project leader and program manager of biopharmaceutical cGMP manufacture from pre-clinical to clinical phase I, II, and III.

At Sartorius, Fern supports customers through incorporating high-throughput technologies into cell line and process development, focusing on Sartorius's Ambr® technology and integrated platforms.

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Katy is part of the Marketing Communications team at Sartorius, where she supports the creation of a variety of written pieces, from published articles to web content.

Before joining Sartorius in 2021, Katy was employed as a Post-Doctoral Research Associate at the University of Edinburgh, where she also completed her doctoral studies. Here, she carried out research in genetics and cellular biology and began taking on writing projects, eventually entering into a career as a freelance writer for various biotech companies and agencies.

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