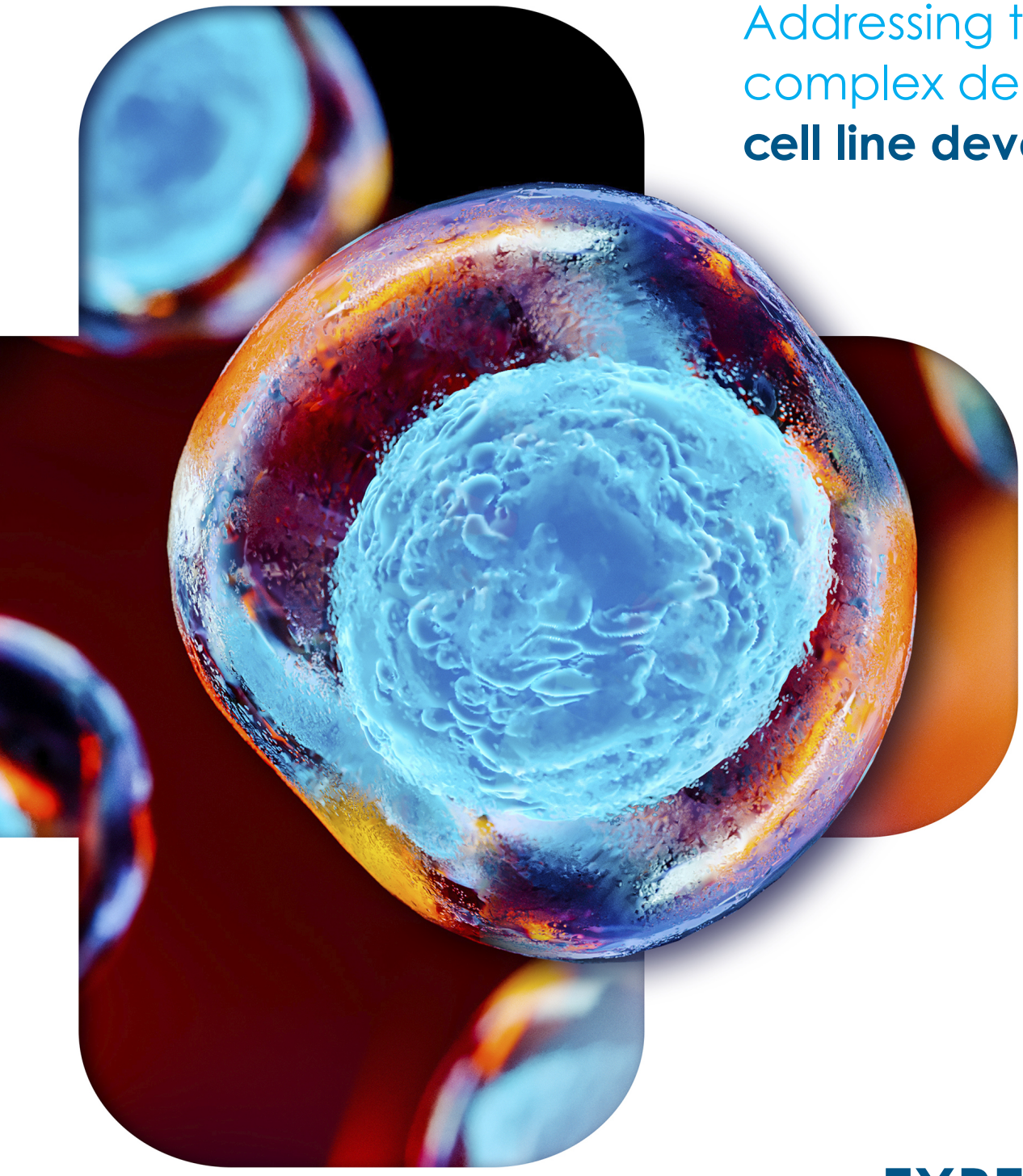
Bora Biologics



Addressing the complex demands of **cell line development**

EXPERIENCE
accelerates success

www.borabiologics.com

Introduction

The global biologics market is growing. Valued at $461.74 billion in 2022, the market value is forecast to increase at a compound annual growth rate of 10.3% from 2023 to 2030[1]. This is due to the promise biologics have shown as treatments for various chronic diseases such as hemophilia A and B, muscular dystrophy, various cancers, and rheumatoid arthritis[2].

To produce biologic drug substances (DS), the genetic material encoding the desired product is inserted into cells and cultured, with the product then isolated, purified and formulated. Harnessing living organisms is an effective method for constructing these complicated materials. Cellular machinery can help assemble numerous individual components efficiently and in the correct order, like forming a protein from amino acid building blocks. Additional modifications to the molecule often occur within the cell before it is isolated for further processing. Biologics are complex and host systems are an effective tool for synthesis, with techniques used to tailor and optimize the organism for the production of the desired DS.

[1]https://www.grandviewresearch.com/industry-analysis/biologics-market

[2]https://www.fda.gov/vaccines-blood-biologics/development-approval-process-cber/biological-approvals-year

Section 1:

A CLD strategy

Cell line development (CLD) is the process of choosing and optimizing a cell line for yield and isolation of a target, and can be broken down into:

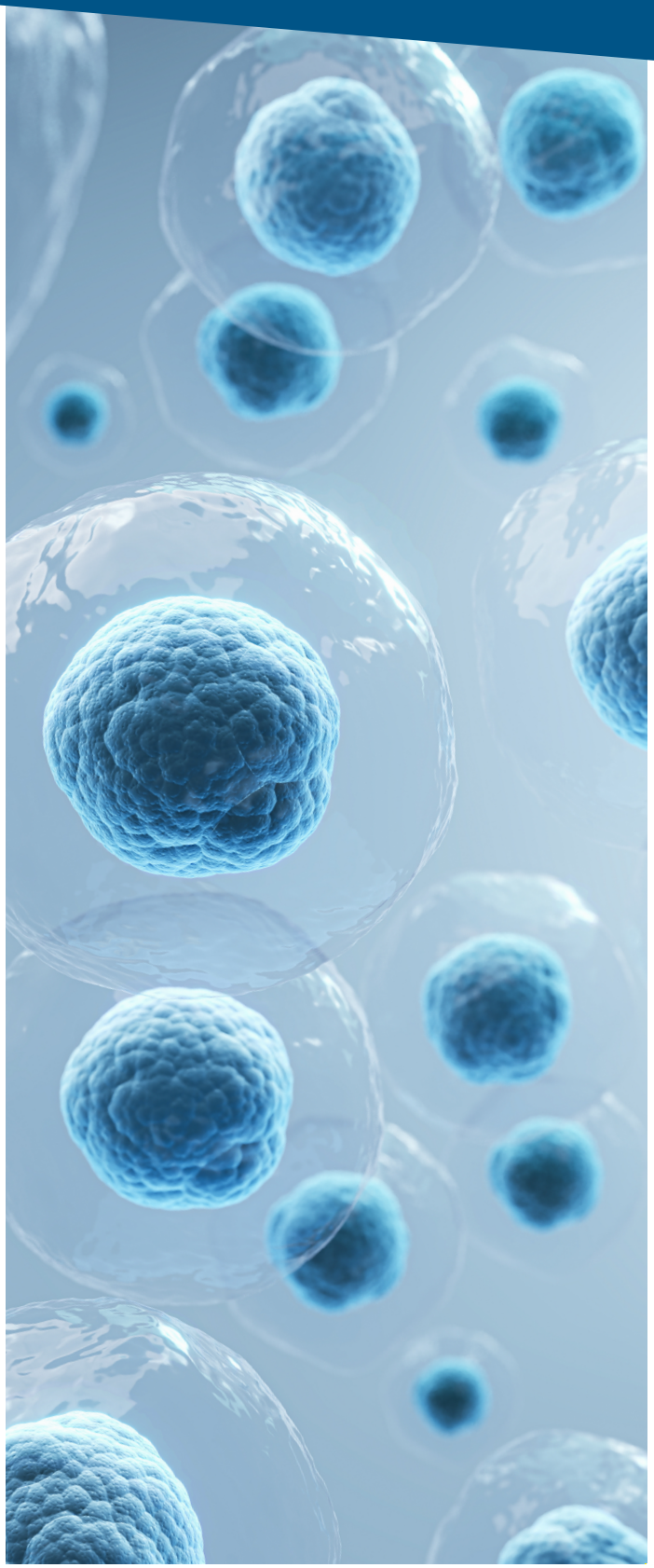
Selection: As every cell line has distinct characteristics that can be beneficial or detrimental to biologic production, deciding upon a cell line that is compatible with the product and aligns with the project goals is essential. Cells are grown and screened, with the most suitable cell lines selected for further analysis.

Single-cell cloning: A single cell from the chosen clones — with high expression, purity and reliability — is isolated and explored further. Ensuring each cell line originates from a single cell limits heterogeneity, which is the variation of genetics within a cell population. This can lead to differences in cell growth and expression levels, potentially changing the characteristics of a biologic. Monoclonality assures that cell lines are genetically similar and that DS production remains consistent and reproducible. The use of single-cell printer technology allows the screening of thousands of clones at a time.

Ranking of clones: The selected single-cell clones are screened and ranked based on favorable characteristics such as productivity, stability and growth.

Lead clone selection: The ranked clones are evaluated, with additional testing of specific parameters relevant to the project at hand. The “lead clone” is identified from these results. Following this, iterative optimization steps aim to instill advantageous characteristics for high-quality target production that can be scaled up for biopharmaceutical production.

Once CLD is complete, the host organism should produce the target DS in high yield, with desired stability and purity. Reaching the point when a cell line is ready for scale-up can be complex, with several challenges that must be addressed for CLD success.



Section 2:

CLD pressures and challenges facing biopharma developers

An effective cell line is an essential part of any biologic manufacturing project. To maintain a competitive advantage in the biopharmaceutical market, there is a demand for speed to bring critical therapeutics to market – as many innovators are chasing the same targets and in the case with biosimilars with the originator organization holding exclusivity until a patent expires. As biologic production is reliant on a robust and reliable cell line, having an effective CLD strategy in place is vital for streamlining production and should consider the following:

The need for speed:

A proactive approach helps to identify potential risks early in the development process, with preventative measures employed to mitigate them. This allows projects to reach key milestones at accelerated rates, reducing the risk of delays as a result of unexpected challenges. Developers need to know where to take risks and where to avoid risk in order to keep on track and also must know how to use the analytical and process development tools at their disposal to ensure that the molecule's integrity is maintained throughout the process.

Achieving high yield:

To maintain a competitive advantage, the process must also be cost-effective. With high product yields, large concentrations of DS can be isolated in limited bioreactor volumes. This also decreases timelines, with large volumes of biopharmaceuticals isolated from fewer or smaller cultures. Engineering the cell line to produce increased product yields is also critical for building a scalable process. This can be done through genetic engineering or through identifying and optimizing cell culture conditions that promote soluble expression.

Regulatory pressure:

Patients' health is a priority, with several measures in place to ensure all DSs are efficacious and safe and ready to be vialed into drug substances (DS) for distribution to clinical sites for patients. These standards are defined by good manufacturing practice (GMP) guidelines, which must be followed when developing a product intended for patient use. For biologic products, which are often limited to parenteral delivery due to their sensitivity to the harsh conditions of the body's natural defenses, these regulations become even more stringent. GMP guidelines require quality control (QC) measures to be in place throughout manufacture. These QC methods ensure a product meets the pre-defined quality standards for regulatory approval.

Having an effective CLD strategy in place is vital for streamlining production

Monoclonality and safety:

With significant impacts on product efficacy and safety, ensuring monoclonality is essential for the manufacture of high-quality products that meet QC standards. Failure to instill monoclonality leads to heterogeneous production. Variability of cells can also be a result of differing growth conditions or materials, in addition to cell line composition. Therefore, when constructing a robust cell line, strict growth conditions are also essential for consistency.

Cell Line stability:

The cell line must also maintain integrity of production of the target protein over a specified number of passages. This ensures that the cell line can be continuously productive over long periods of time. This is especially important if scaling up to very large bioreactor volumes or in perfusion systems.

Section 3:

A robust CLD approach meets biologic needs and overcomes challenges

As it can greatly impact product quality, quantity and reproducibility, choosing an appropriate cell line is the first step of CLD. A number of cell lines have been engineered for biologic manufacture, but the use of Chinese hamster ovary (CHO) cells remains a popular choice, with over 70% of recombinant proteins produced using this line. The following advantages position CHO cell lines as a favorable option:[3]

Closely conserved with nature: Post-translational modification and folding of proteins are closely conserved with human proteins and so are more likely to be accepted by the human immune system.

Robust: Tolerant to changes in pH, oxygen levels, pressure or temperature throughout manufacture, CHO cell lines allow production systems to be modified to suit the target DS.

Easily scaled: These cell lines are compatible with growth in serum-free suspension cultures with high cell concentrations, which is the preferred method for large-scale bioreactors, easing scale-up.

Well-understood and characterized: Extensive literature is available, which can provide key insight and help accelerate timelines for approval.

With a regulatory track record: Previous authorization of CHO-produced materials streamlines the FDA approval process with respect to CMC activities.

Despite the advantages, a cell line still needs to undergo a rigorous optimization process to introduce the desired characteristics for the efficient manufacture of the target biologic. Both genetic engineering and non-genetic optimization solutions can be used to adapt the cell line culture to the product at hand.

When producing a biologic, the genetic material must first be inserted into the cell. Several methods can be used to integrate the vector encoding the DS into the cell, including viral, chemical and physical transfection techniques. The selected method should limit variability and be compatible with both the vector and the cell line, leading to uniformity[4]. Cell enrichment methods then identify and isolate the cells with the most beneficial characteristics. The use of high-throughput screening (HTS) techniques — such as a high-resolution imager or state-of-the-art single-cell printer — allow for a large number of cell clone "units" to be screened, ensuring monoclonality.

Manipulation of culture conditions can be an effective strategy to increase expression levels of materials with low productivity. Screening of pH, temperature and nutrient composition of the culture media is key to identifying the optimal growth conditions. Additional components can also be added to the media to drive expression and promote product solubility. For example, the addition of resveratrol and insulin growth factor-1 to CHO cell lines in serum-free media resulted in increased growth[5].

A scalable platform allows for flexibility, enabling changes in capacity in response to changing market needs. Engineering a cell line with the ability to adapt positions the production line for success and ensures that no resource or expense is wasted.

[3]Dumont, J., Euwart, D., Mei, B., Estes, S., & Kshirsagar, R. "Human cell lines for biopharmaceutical manufacturing: history, status, and future perspectives." Critical Reviews in Biotechnology, 36:6, 1110-1122, (2016). https://doi.org/10.3109/07388551.2015.1084266

[4]Chong, Z. X., Yeap, S. K. & Ho, W. Y. Transfection types, methods and strategies: A technical review. PeerJ 9, (2021). https://doi.org/10.7717/peerj.11165

[5]Li, W., Fan Z., Lin Y., and Wang T. Y. "Serum-Free Medium for Recombinant Protein Expression in Chinese Hamster Ovary Cells." Frontiers in Bioengineering and Biotechnology, 9, (2021). https://doi.org/10.3389/fbioe.2021.646363

Section 4:

The importance of analytics in CLD

Biologic analytics are essential throughout drug development to ensure that a product remains stable and active throughout. The complex nature of biologics and their reliance on parenteral delivery methods means regulations are more stringent. As defined by the GMP, biologic materials must meet distinct critical quality attributes (CQA), proving that product activity, purity and potency meet regulatory requirements. These CQAs need to be met throughout development and manufacturing. Analytics are leveraged throughout to monitor and quantify production to meet efficacy and quality standards.

Woven into development and manufacture, analytics are utilized to characterize cell lines, screen and select components based on specific characteristics, help guide process optimization and provide proof of regulatory compliance. Implementing these analytical methods early in development allows for tailored development and manufacturing.

With the full characterization of the biologic leads to fully understanding the molecule, for example. characteristics such as stability can be determined. The manufacturing method can be designed with this insight, tailoring the techniques to infer stability throughout production, avoiding any methods where the product has high susceptibility to degradation and aggregation. Delays as a result of incompatibility with the DS can also be avoided. Formulation with knowledge of characterization means compounds that are likely to interact with the biologic will not be used, helping to inform formulation development and streamlining the process.

Section 5:

Tactics enabling analytical success for CLD

Identifying and implementing analytical techniques to get the most information at the best stage throughout development and manufacture can be difficult. Having a tactic to approach this is a key driver of analytical success:

Choosing appropriate bioassays:

Assays are an effective technique to monitor in-process material uptake or release, characterize a product or measure stability. Selecting and optimizing an assay for a desired purpose is complex. The ability of an assay to mimic the MOA is essential and designing the assay for a specific role at a particular point in development and manufacture can be invaluable in providing information to inform future development and manufacturing stages. The intricacy of assay development does make optimization, performance and maintenance expensive. Chosen depending on the product and process, common bioassays such as enzyme-linked immunosorbent assays (ELISA), surface plasmon resonance (SPR) techniques and cell-based options can be used throughout. Cell-based bioassays as potency release assays are important for Phase II and beyond.

Introducing more sensitive testing:

Introducing methods with very low limits of detection and high sensitivity can significantly reduce the amount of product used during analytical testing, limiting product loss and reducing costs.

Understanding product characteristics:

Leveraging analytics to understand product characteristics early within development can drive CLD, allowing optimization of key attributes (such as stability) in line with target needs. Processing steps further down the pipeline can be tailored to the product to infer stability, streamline production and mitigate risk. This understanding can also inform formulation development to meet desired characteristics for patient needs and regulatory standards.

Transparent communication with specialists:

Outsourcing analytical testing is common within the biopharmaceutical industry. Ensuring free-flowing communication can drive analytical efficiency, providing key insight into product stability and activity to better understand and tailor analytic methods to the product and required CLD at hand. In addition, communication of expectations and timelines ensures that milestones are met timely and that analytic development runs smoothly.

Following these techniques to implement an effective analytical development strategy can circumvent the need for extensive and complex formulations post-production to repair the protein. In combination with coordination and free-flowing communication, this communicative and proactive relationship helps to ensure meticulous characterization and top-level visibility, which is especially important for emerging types of molecules where less information is known.

Section 6:

CLD case study

Leveraging Bora's CLD expertise to commercialize a novel blood diagnostic device

Bora Biologics was approached by Q-Sera to help with the development and manufacture of RAPClot™ (ecarin), a component of the coating solution used on tubes for high-quality serum samples. RAPClot™-based tubes have been designed as an alternative to currently used blood-collection tubes (e.g., silicon-based). Upon collection, samples rapidly clot to produce a high-quality serum for analysis. A cost-effective method for sample preparation, RAPClot™ tubes will also allow patients to receive accurate and quick results post-analysis.

Ecarin is a large protein found in some snake venoms that must be cleaved to form the active component. Grown in mammalian cells, initial low yields meant that production of this compound was not commercially viable. To compete with the market value on unit price and production cost compared to current coated tubes, manufacturing needed to be more cost-effective. This led to Q-Sera seeking a partner in Bora, utilizing our dedicated cell line team and expertise in early-stage CLD to increase the yield of RAPClot™.

Using an effective CLD strategy, Bora helped Q-Sera with the following steps:

Proof-of-concept study: Using its proprietary CHO cell lines, early CLD platform data showed increased productivity compared with the customer's original clone. Selecting the lead clone, further optimization was completed.

Leveraging platform for productivity: The technical team tailored CLD and cell culture processes to stabilize and streamline production while enhancing the yield and quality of RAPClot™. An evident productivity improvement of 5x was observed, leading to yields with a 10-15% increase when a more efficient activation step was used, and reducing costs.

Improved downstream efficiency: Additional optimization aimed to improve steps further down the development pipeline. Reduction of the number of ultrafiltration (UF)/ diafiltration (DF) steps between chromatography and minimizing the number of chromatography columns from three to two streamlined the production process. Utilizing only two chromatography columns not only increased product quantity but also led to higher purity.

Using a robust CLD strategy and harnessing analytics throughout, through Bora's expertise and experience in CLD, the best-performing clone was selected and production of the complex RAPClot™ protein was optimized with a streamlined process for a more cost-effective and competitive approach.

How can Bora Biologics help to support your next biologics project?

Having a robust CLD strategy is the foundation of biologic production that can propel a project, helping to streamline production while mitigating risk. A tailored and optimized cell line can be instrumental in overcoming development and manufacturing challenges and achieving a competitive advantage in the market with streamlined manufacturing processes.

Bora Biologics is a contract development and manufacturing organization (CDMO) with expertise and proven experience in advancing biologic projects through effective CLD methods. Providing access to our proprietary cell lines, high-throughput technology and a team of experts, Bora Biologics can help drive your CLD project forward.

To find out how we can help you succeed in your next CLD initiative, **contact us today.**