Whitepaper: The power of partnership in advancing innovation

Sutro Biopharma and Boehringer Ingelheim collaborate to scale-up a novel cell-free technology platform to produce ADCs

Introduction

The biopharmaceutical industry is witnessing a significant transformation as it adapts to an expanding market and a growing demand for treatments that address unmet medical needs. Among the various innovations, antibody-drug conjugates (ADCs) have emerged as a noteworthy development, offering a more targeted approach to cancer therapy. ADCs combine the specificity of monoclonal antibodies with the potency of cytotoxic drugs, aiming to selectively target cancer cells while minimizing impact on healthy tissue. This specificity could lead to treatments with better efficacy and reduced side effects, representing a substantial improvement over traditional chemotherapy.

Figure 1 shows that the ADC market has witnessed significant growth in recent years, driven by advancements in linker technologies, payload optimization, and antibody engineering. It is projected to grow at a compound annual growth rate (CAGR 2024-2030) of over 20%, reaching several billion USD by the end of this decade. This growth is fueled by the increasing number of ADCs entering clinical development and regulatory approvals. As of 2025, there are over 15 FDA-approved ADCs, including landmark therapies such as trastuzumab deruxtecan and brentuximab vedotin, which have set the stage

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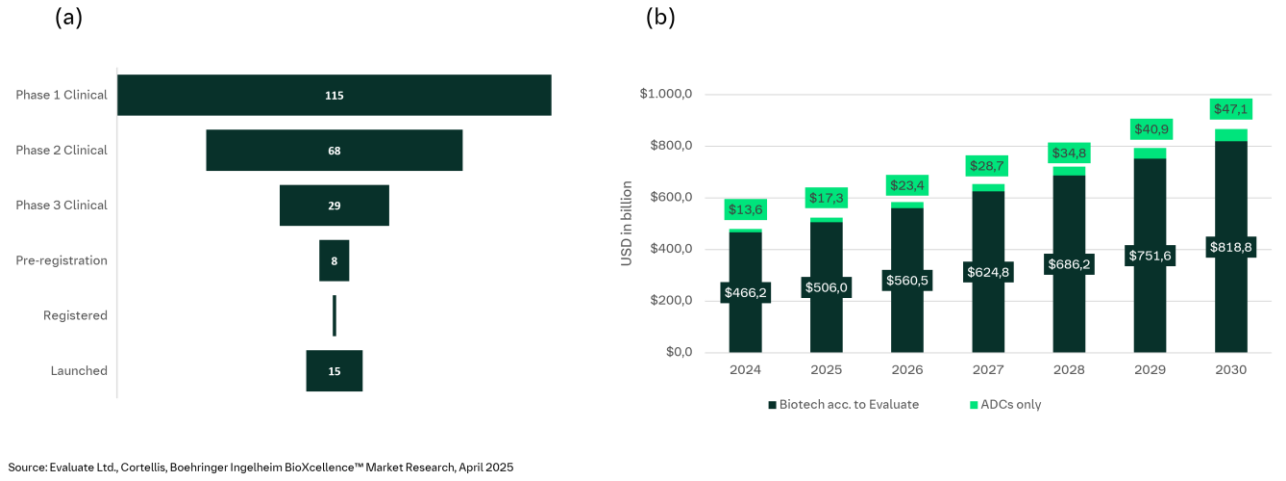
Life forward

for subsequent innovations. The expanding pipeline reflects the increasing interest in ADCs across both academia and industry, with a focus on addressing unmet medical needs in oncology and beyond for the benefit of patients.



Source: Evaluate Ltd., Cortellis, Boehringer Ingelheim BioXcellence™ Market Research, April 2025

Figure 1: (a) The ADC pipeline is richly filled. Currently, there are approx. 250 ADC therapies in different clinical phases or launched worldwide, of which 15 products have been launched in major markets. (b) As the global sales forecast reflects, among the attractively growing entire market of biopharmaceuticals, ADCs experience an even more enhanced CAGR of more than 20% (2024-2030). This demonstrates the growing significance these medications have for patients.

ADCs have the potential to be tailored to combat a variety of cancers, which adds to their appeal in the oncology field. However, their production presents unique challenges. These biological compounds are made up of three components: the antibody, a linker, and the payload. Each part must be carefully synthesized and connected, which requires precision and stability throughout the process. The complexity of ADCs extends to their manufacturing, which is inherently tied to living cells. Variations in cell-based production can significantly affect product quality due to the dependence on consistent cell output. Thus, consistency in the quality of cell output is crucial, as it directly impacts the final product's quality.

Traditional cell-based methods are further limited by the lifespan and productivity of the cells themselves. The variability in the biologic products they produce can complicate quality control, safety, and regulatory compliance, making manufacturing a delicate balance of craft and science.

This whitepaper will delve into the evolving biopharmaceutical landscape, with a focus on the manufacturing challenges and advancements associated with ADCs. It highlights the strategies being employed to overcome these challenges, setting the stage for ADCs to become a more common option in cancer treatment. We will examine the hurdles faced in the large-scale production process to prove commercial viability. Through this exploration, we aim to present a clear picture of the current state of ADC development and the path forward towards commercialization in this dynamic sector of medicine.

A novel production route: cell-free expression of therapeutic proteins

Addressing the complexities of ADC manufacturing, Sutro Biopharma (Sutro) has pioneered a cell-free protein synthesis platform that revolutionizes the traditional production process. This innovative approach leverages cellular components necessary for protein generation outside of the living cell

environment. Although the first thoughts and research at Stanford University (USA) on cell-free expression of therapeutic proteins were initiated 30 years ago, Sutro and Boehringer have only now proven its commercial viability.

> Is it possible to harvest the internal machinery of *E. coli* and use it in a biochemical reaction, free of living cells, to cost-effectively manufacture therapeutic proteins?
>
> J. Swartz | Stanford University, in the 90ies

Figure 2: The first thoughts and research on cell-free expression of therapeutic proteins evolved 30 years ago, but it took some while to turn this innovation into a reality for patients.

The cell-free extract, replete with the machinery for energy production, transcription, and translation, is primed to synthesize proteins upon the introduction of a specific DNA sequence. This method effectively bypasses the challenges of cell culture, enhancing predictability and streamlining production. Furthermore, the cell-free platform can produce protein in just approximately one week. This is considerably faster compared to the 2-3 weeks required to manufacture an antibody using CHO cells. This rapid production capability 'fast-tracks' the creation and evaluation of target proteins, enabling the swift screening of potential molecules.

Sutro's proprietary technology harnesses the cell's intrinsic transcription and translation systems to create specific polypeptides at the ribosome and even supports the incorporation of non-canonical amino acids. This process, occurring outside the confines of a cell, allows for the production of antibodies or any desired protein with remarkable control. Additionally, the platform provides insights into the production process, enabling the optimization of molecule designs to boost production yields or enhance product quality.

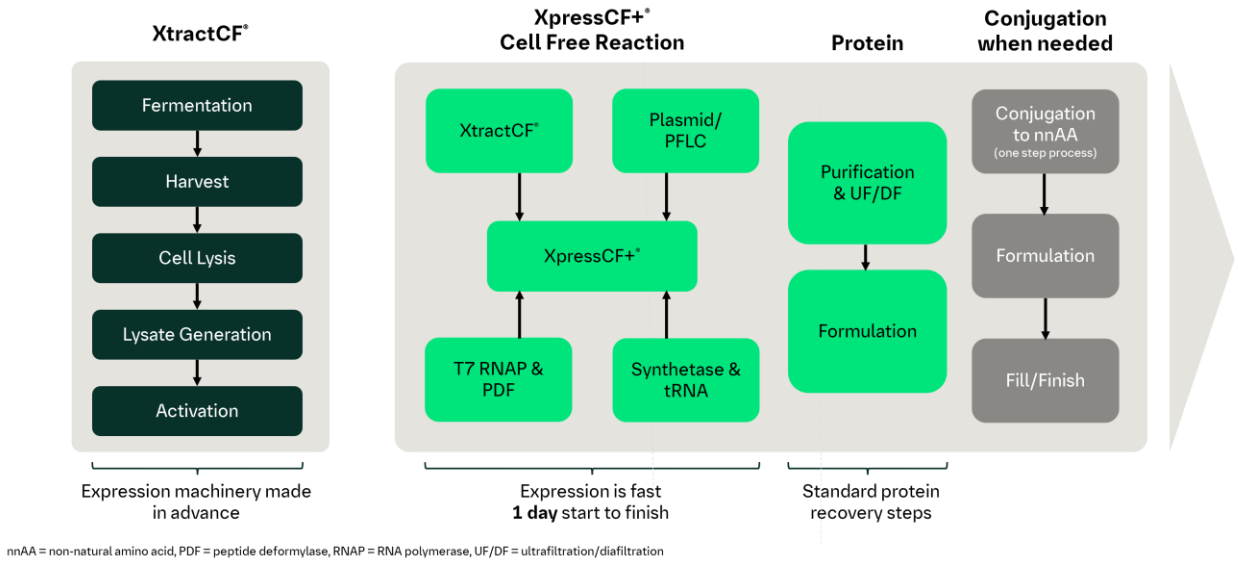


Figure 3: Based on the expression machinery XtractCF® delivered by Sutro, at Boehringer Ingelheim the Antibody Intermediate (AbI) is produced in a cell-free reaction, followed by protein purification and formulation steps (accent green). The drug conjugation is performed by a third party.
XtractCF® = solution containing the expression machinery derived in pre-step, XpressCF+® = conjugation technology that relies upon incorporation of a non-natural amino acid that enables click chemistry conjugation between a polypeptide and a small molecule, nnAA = non-natural amino acid, PDF = peptide deformylase, RNAP = RNA polymerase, UF/DF = ultrafiltration/diafiltration, PFLC = pre-fabricated light chain.

A key advantage of Sutro's platform is the ability to rationally design ADCs with extreme precision. The technology facilitates the strategic engineering of conjugation sites, ensuring that the location and impact of the conjugation are meticulously controlled. This level of precision is not just a technical achievement but has significant therapeutic implications.

The incorporation of non-natural amino acids through the cell-free technology is another breakthrough, allowing for site-specific conjugation. This capability enables a 'mix and match' approach to payloads, varying their locations on the antibody and their quantities. This flexibility is a crucial element in the creation of next-generation ADCs, which are designed to show improved safety and efficacy profiles compared to those produced by traditional methods.

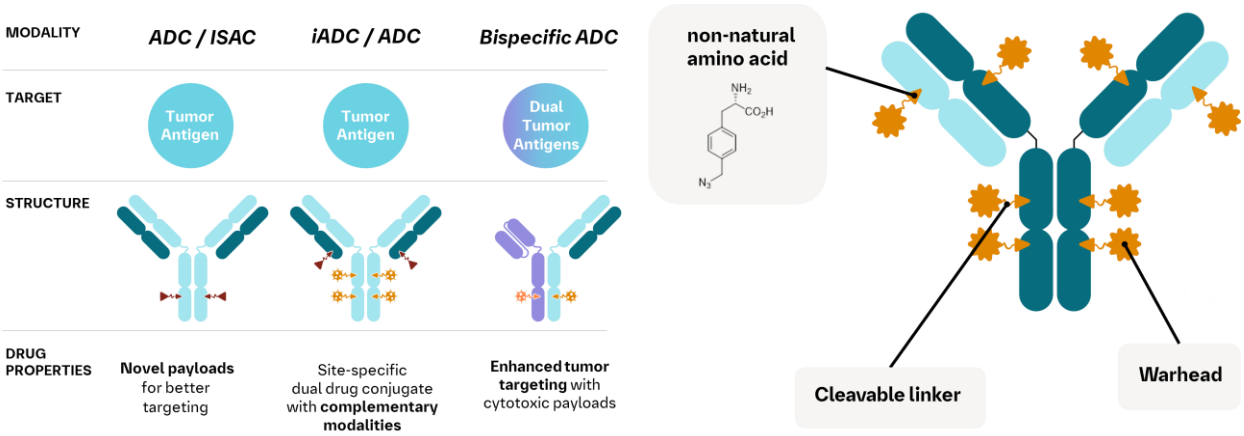


Figure 4: Sutro's technology enables novel ADC structures, e.g., immunostimulatory antibody drug conjugates (iADCs). © Sutro Biopharma

Manufacturing antibody-drug conjugates at a commercial scale

Scaling-up the manufacture of antibody intermediates to a commercial level is a complex endeavor that requires specific capabilities. "One of the most compelling aspects of Sutro's technology is its scalability. To complement our internal expertise, we actively seek partnerships that can strengthen our platform and elevate it to the next stage of maturity – delivering large-scale patient supply for clinical studies and commercial markets," says Venkatesh Srinivasan, Ph.D., Chief Technical Operations Officer, Sutro Biopharma. The same system that facilitates discovery and research can seamlessly shift into development and large-scale manufacturing. This continuity ensures that the transition to commercialization is efficient and consistent, maintaining the integrity of the product throughout its lifecycle.

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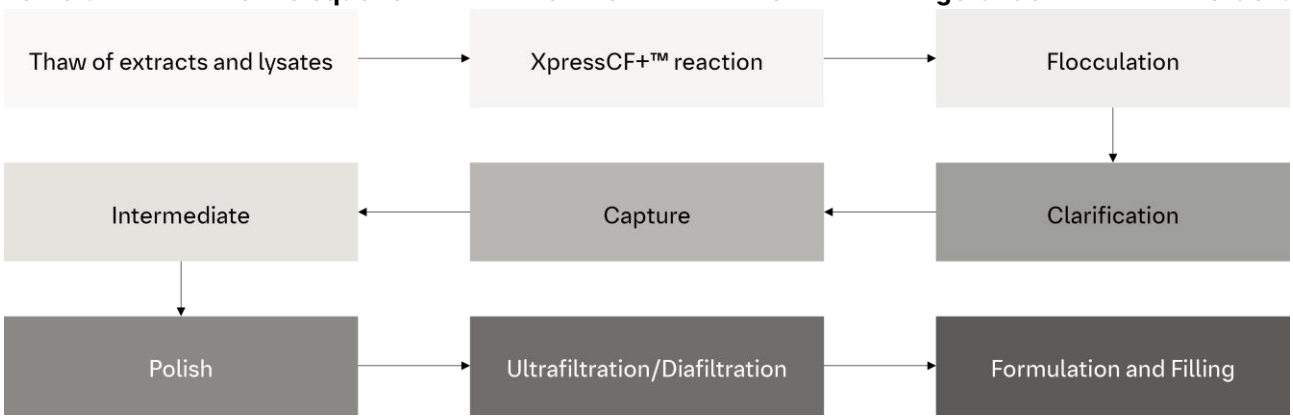

Figure 5: Process chart of the cell-free expression production process of monoclonal antibodies at Boehringer Ingelheim's biopharmaceutical manufacturing facility in Vienna.

Overcoming manufacturing challenges requires innovative solutions and flexible approaches to accommodate the production process, which is depicted in Figure 5 at a high level. A significant challenge involves the logistical complexity of combining several critical raw materials at a specific point after the

thaw process for the subsequent cell-free reaction (see Figure 3 and Figure 5). Managing hold times at the appropriate temperatures of the sensitive materials is vital.

The bioburden load of extracts and lysates, which are of biological origin and are main components in the manufacturing process, is a critical factor. Cell extracts and most lysates are challenging or impossible to filter due to the critical components they contain, which are essential for the process. For bioburden control, closed and aseptic handling within the plant is crucial. Typically, for classical microbial processes, all materials are usually either autoclaved or filtered before being transferred into the bioreactor.

A high level of operational excellence is a prerequisite for the aseptic processing of extremely high solution volumes (hundreds of liters) from different sources in a short time. Addressing this challenge requires out-of-the-box thinking and the ability to react with great flexibility to the dynamic manufacturing environment.

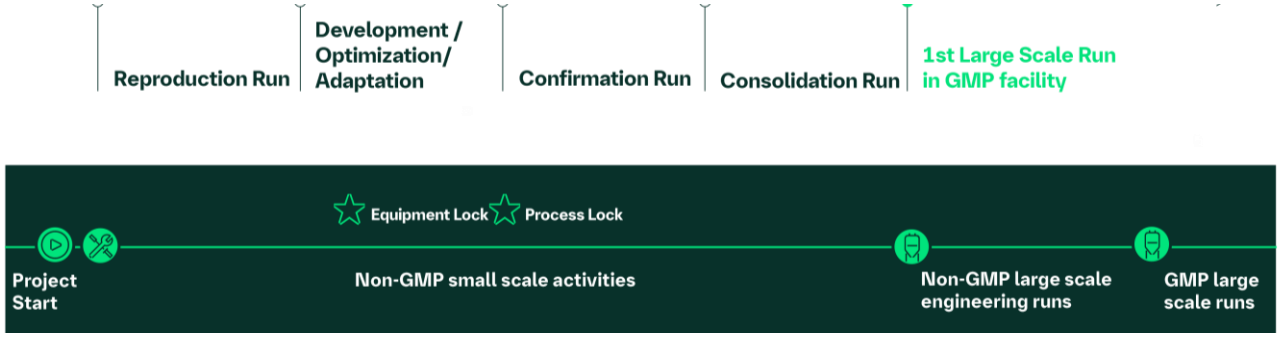

Figure 6: Process transfer approach at Boehringer comprising reproduction runs at small scale to identify facility fit adaptations, followed by confirmation and consolidation runs to prove reproducibility in pilot scale and final technical implementation in non-GMP large-scale facility with engineering run.

According to our process transfer concept as shown Figure 6, the process was first reproduced at small scale (so called reproduction runs). Multiple challenges appeared when transferring the process into Boehringer Ingelheim's microbial facility in Vienna, mainly because the process was initially developed for manufacturing in small scale single-use bioreactors. It was then agreed upon that facility fit adaptations were needed to integrate the process into the large-scale multi-product plant. These adjustments were carried out during the adaptation/optimization phase, which included, e.g., adjustments for dissolved oxygen and the gassing strategy.

After adaptation, the final process was consolidated at pilot scale to prove the reproducibility of the modified process.
The next step was large-scale manufacturing: the process was technically implemented at scale in a 4k L manufacturing plant via an engineering (ENG) run first. Later the successful ENG run was followed by two successfully completed 4k L GMP runs. The material out of both GMP runs could have been fully released for clinical phase III supply. Refer to Figure 7 for a visual comparison of bioreactors at various scales.

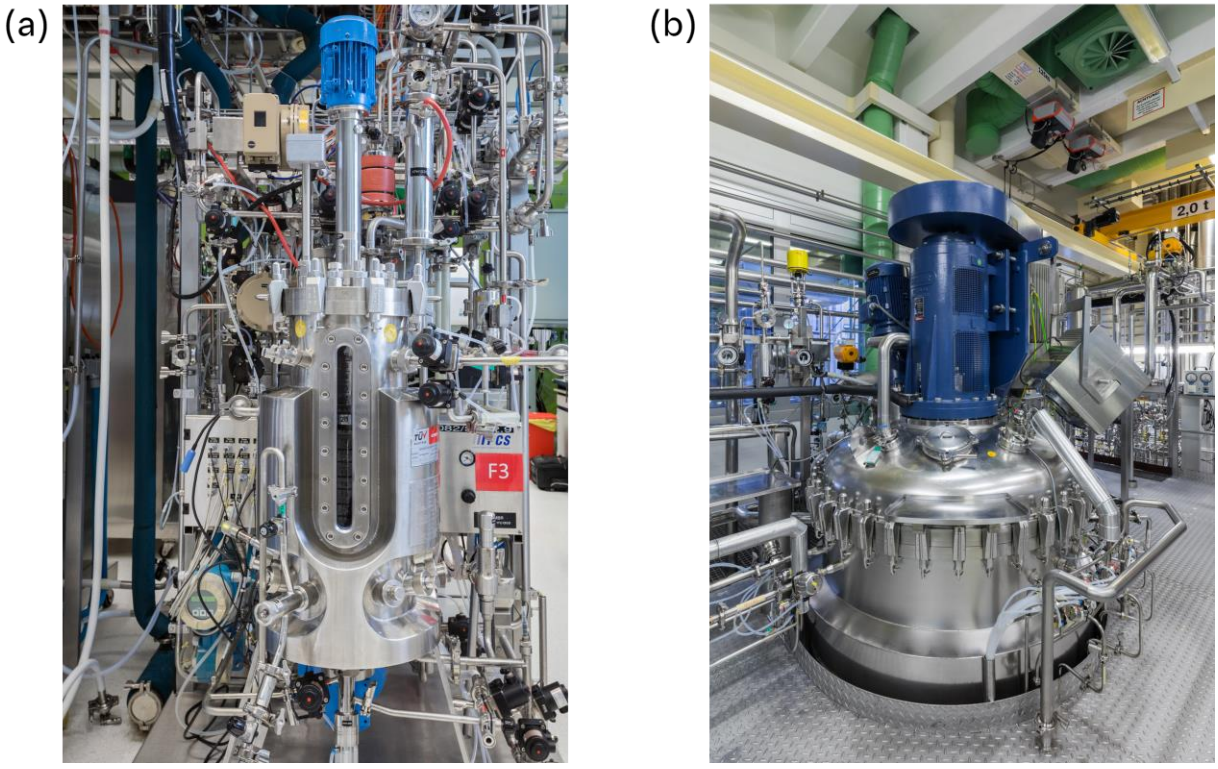

Figure 7: Bioreactors with different fermentation volumes, namely 20 L (a) and 4,000 L (b), were used to demonstrate the scalability of the cell-free expression reaction.

An essential aspect of this process transfer and the associated facility fit adaptations was the management of dissolved oxygen and the development of an effective gassing strategy. Originally developed by Sutro for use in cell culture single-use bioreactors, the process acknowledges that oxidation can adversely affect product quality. It is therefore crucial to maintain a balanced level of oxygen necessary for the reaction while simultaneously avoiding oxidation.

This balance is particularly challenging because microbial bioreactors, designed for very high oxygen transfer and agitation speed to ensure fast oxygen distribution, operate differently compared to cell culture bioreactors. To address this, a new gassing strategy was successfully developed. This strategy not only meets the specific process needs but also seamlessly integrates into the existing microbial stainless steel multi-product plant, as the comparison of dissolved oxygen profiles in Figure 8 shows.

Engineering and the first GMP runs revealed process titers comparable to those observed during the consolidation runs at the 20 L scale, confirming the successful scale-up of the process by a factor of 200 (see Figure 9Figure 9).

By innovatively addressing these challenges, it was possible to enhance manufacturing efficiency and ensure the production of high-quality products. Product quality could not only be met, but even exceeded for several quality attributes, as demonstrated in Figure 10 Figure 10Figure 10. Quality control analysis revealed a significant alignment of parameters across various manufacturing runs, with all product

specifications successfully met. The titers were maintained or even slightly improved during GMP large-scale production, while consistently achieving high product quality (as illustrated in Figure 9 and Figure 10Figure 10). The kinetics of product production as well as the purity and impurity profiles were consistent across all production scales. Even a slight learning curve was observed throughout the process, with some quality parameters showing measurable improvements over time.

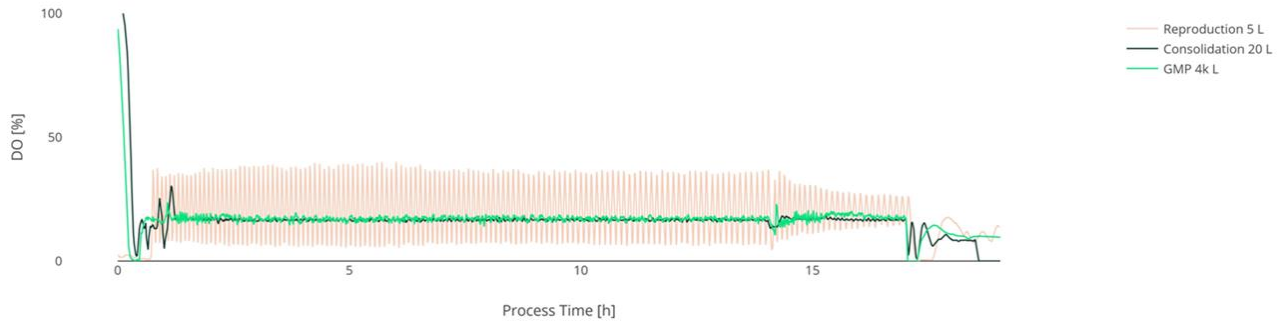

Figure 8: Comparison of dissolved oxygen (DO) profile between first reproduction run (5 L), final consolidation (20 L pilot scale) and 4k L GMP run.

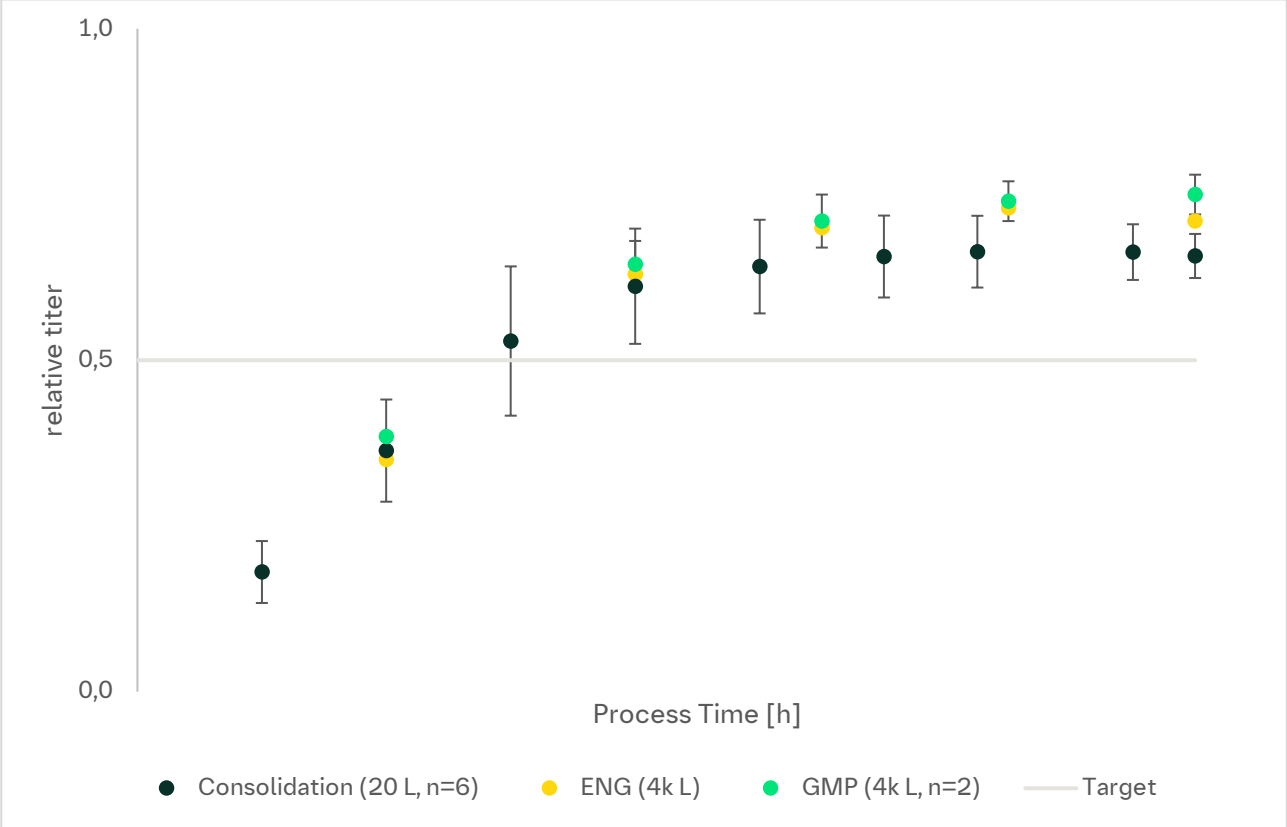

Figure 9: Comparison of titer production upstream between final consolidation (20 L), ENG (4k L) and GMP (4k L).

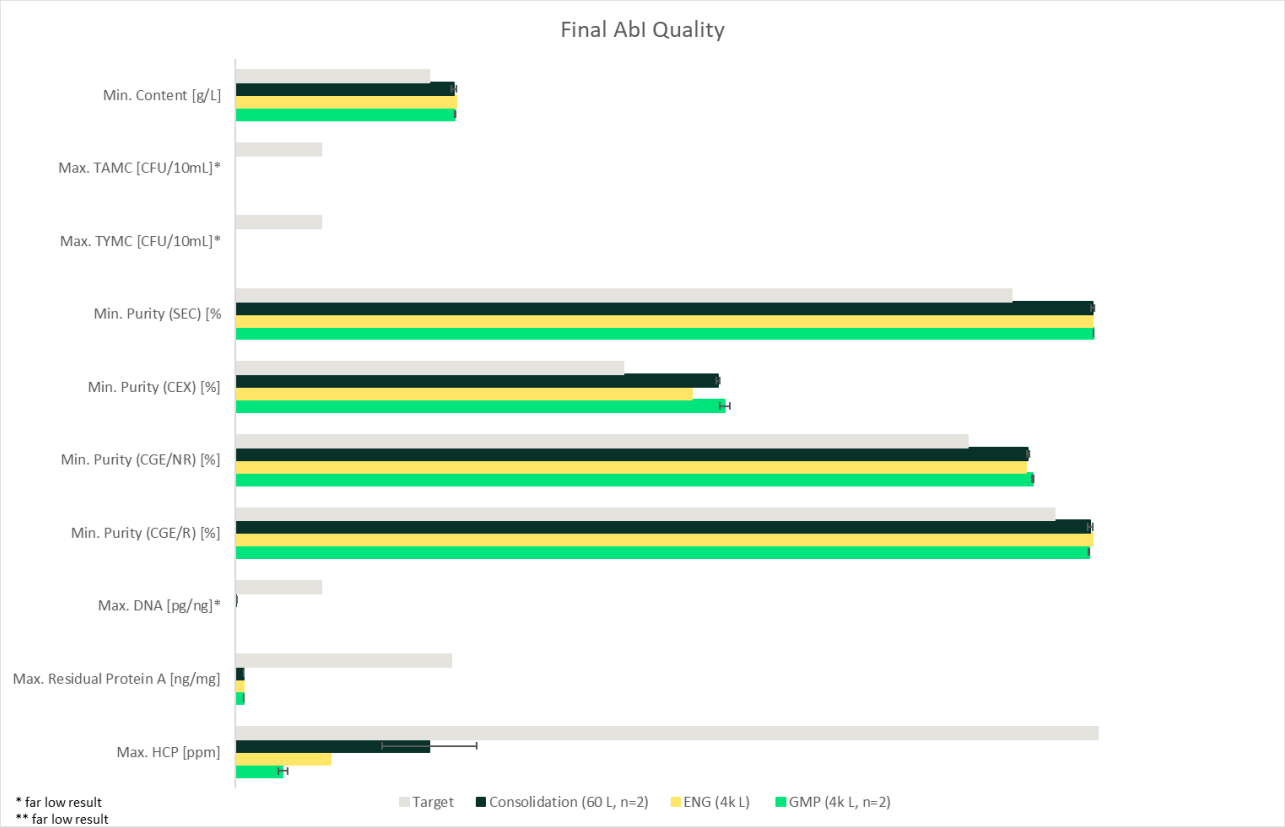

Figure 10: Comparison of final AbI product quality between final consolidation (20 L), ENG (4k L) and GMP (4k L). TAMC = total aerobic microbial account, TYMC = total combined yeasts/moulds count

Conclusions and summary

Sutro's proprietary cell-free XpressCF+® platform enables the efficient production of unique ADC constructs that are not achievable with other methods. This allows for precise tailoring of therapies to optimize clinical outcomes and enhance patient experience. The platform and approach aim to optimize all aspects of ADCs, including payload type, linker, linker positioning, drug-to-antibody ratio, and antibody design.

The objective of this optimization is to address the limitations of ADCs by ensuring that the drug, typically a chemotherapy agent, is delivered to and released at the targeted cells, thereby minimizing the impact on healthy cells.

By combining the unique cell-free XpressCF+® platform with proven manufacturing capabilities, Boehringer is positioned to deliver a consistent and efficiently produced drug, addressing a common challenge in the ADC space.

Sutro's lead candidate was selected to demonstrate commercial viability, providing insights that can be applied to their pipeline of next-generation ADCs targeting various cancers. Current ADCs are effective,

but their inherent toxicity limits dosage – what makes them active in the tumor also affects cells outside the tumor. Sutro's proprietary platform is capable of maximizing the therapeutic benefits while potentially offering a better safety profile. Their ADCs are designed to be gentler outside the tumor, allowing for increased dosages, longer treatment duration, and improved clinical outcomes. The technology is particularly suited for designing, discovering, and producing more complex ADCs, including novel dual-payload ADCs.

One and the same approach is used from early research to commercial scale and works. The facilities and equipment as well as quality and operation systems are already in place for successful scale-up to commercial volumes in facilities using traditional tools and platforms. Thus, Sutro's cell-free platform is not capital intensive.

Sutro and Boehringer Ingelheim have successfully tackled new challenges in producing commercial quantities of cell-free ADCs for the first time. Much of this effort was focused on securing the cold chain for supplying the required quantities of raw materials while maintaining required temperatures. Additionally, targeted process adaptations to ensure facility and process fit were implemented, showcasing our expertise and experience in operational excellence as well as exemplifying great collaborative teamwork to find solutions when addressing challenges jointly with partners.

While Sutro is actively pursuing business development opportunities to maximize the potential of the platform, Boehringer Ingelheim is the ideal partner for commercial manufacturing, with proven expertise in large-scale supply of this cell-free technology.