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Special Series
Biopharma

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Five Thoughts: Cell Culture Media

Looking at key milestones, challenges, and innovations in cell culture media – and what lies in store ahead.

By Sinan Ozer

Milestones to date

Cell culture media has undergone significant evolution – from simple formulations to specialized and chemically defined compositions. Key milestones over the years include the development of serum-based media, enabling cell growth outside the body. There was later a shift to serum-free formulations, aiming to reduce variability and contamination risks arising from animal-derived components. Further advancements introduced chemically defined media, improving reproducibility and standardization. Recent milestones involve the emergence of specialized media tailored for specific cell types or applications, enabling more precise control over cell behavior and function.

Important considerations

Choosing the right cell culture media includes assessing factors such as cell type, growth requirements, and intended applications. Good media offers optimal cell growth, viability, and reproducibility, and minimizes batch-to-batch variability. It should support desired cell functions and maintain genetic stability.

Choosing inappropriate media can lead to suboptimal cell growth, altered gene expression, or even cell death. This affects experimental reproducibility, leading to unreliable data, prolonged research timelines, and increased costs due to failed experiments.

Common problems

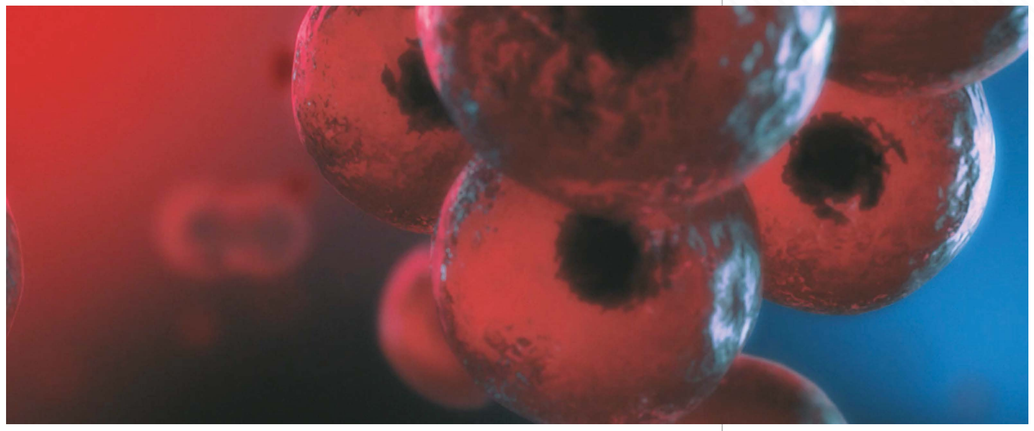
Common mistakes involve neglecting to optimize media for specific cell types, using outdated formulations, or overlooking the impact of media on experimental outcomes. Cells can be cultured successfully by understanding their requirements, regularly optimizing media conditions, staying updated on advancements, and validating media for intended applications.

Other challenges facing drug developers include ensuring media consistency, navigating regulatory complexities, and meeting changing industry standards. And let's not forget the difficulties encountered when scaling up production, overcoming batch-to-batch variations, and developing specialized media for diverse cell types or applications.

Innovations

Many current innovations focus on serum-free, chemically defined media for various cell types, incorporating components that mimic in vivo environments. Advancements include using advanced analytics, machine learning, and bioprocess engineering to develop superior media formulations, improving scalability, and performance. Some drug developers are also shifting from traditional monolayer cell cultures to 3D cell cultures that allow cells to grow in a more physiologically relevant environment that resembles tissue structures. 3D culture offers improved cell–cell interactions and mimics in vivo conditions better for studying complex cell behaviors, drug responses, and disease modeling.

Some companies may seek custom media for unique cell types, either when existing formulations fail to meet precise growth requirements or for logistical reasons related to efficient scaling up. Tailored media can help enhance cell viability, productivity, and functionality, which are crucial for research or production processes.



Credit: Corning Life Sciences

The future

The future of cell culture media involves personalized formulations tailored for specific cellular functions or disease models. Advancements in bioengineering, microfluidics, and organoid technologies may shape media design, allowing more accurate replication of in vivo conditions and enabling precise control over cell behavior and function. Additionally, sustainable, animal-free media could become more commonly used to meet ethical and regulatory demands.

Sinan Ozer is Product Line Manager, Media at Corning

Pushing the Boundaries of Bioprocessing

Biopharma tech continues to advance so why is downstream processing such a headache? Chromatography, in particular, is an expensive process – but improvements are being made.

By Jungmin Oh

Downstream processing in biotechnology and pharmaceutical industries has undergone significant advancements in recent years. We've seen increased adoption of single-use technology, which can reduce contamination risks, lower capital costs, and increase manufacturing flexibility. Additionally, continuous processing has gained traction over traditional batch processing because of its productivity and footprint advantages. Advanced chromatography techniques have also evolved, resulting in higher purity levels, increased throughput, and enhanced efficiency in purification. Furthermore, there's been a focus on process intensification, employing higher capacity resins, multi-column chromatography systems, and integrated process trains. Automation and digitalization technologies have also been integrated to improve process control, data management, and real-time monitoring of critical parameters.

And yet, despite these notable advances, downstream processing continues to present challenges. The complexity of biologics necessitates meticulous purification processes, often involving multiple downstream steps. Companies also need to consider equipment scalability, process transferability, and maintaining quality at larger scales. All of this demands substantial investments in process optimization.

At the same, downstream processing is inherently expensive. Raw materials and consumables can all be pricey – and the costs increase significantly as production is scaled up. Low productivity and yields are also commonplace because of inefficient recovery and purification processes, leading to suboptimal manufacturing efficiency that increases costs even further. Complex purification processes with multiple steps, including chromatography and filtration, require careful tuning to balance purity, yield, and productivity. Ensuring product stability and shelf-life while removing impurities such as host cell proteins and DNA demands robust purification strategies and precise control over storage conditions.

Focusing on chromatography processes

Chromatography is a pivotal technique in downstream processing. Here, the key challenges include achieving sufficiently high selectivity to separate the target biopharmaceutical from impurities (particularly for complex molecules with similar properties) and obtaining adequate resolution between closely related species to ensure desired purity levels (as just one example, consider the problems many biopharma manufacturers now face in separating empty/full capsids of AAV particles).

The limited binding capacity of chromatography columns can hinder throughput and increase processing time, especially in large-scale production, while scaling up processes poses challenges related to column packing and flow dynamics. Chromatography is also an expensive part of the downstream process – the resins, buffers, and the hardware itself can all come with high price tags.

However, chromatography equipment and resin technologies are continuously evolving. Advancements in resin technology are focusing on creating novel stationary phases with improved selectivity, capacity, and stability – enabled by clever chemistry, such as surface modifications and ligand immobilization techniques, tailored to specific biomolecules and purification challenges. Multi-modal and mixed-mode resins integrate various chromatographic functions into a single stationary

phase, providing enhanced selectivity and flexibility to purify complex biomolecules. Continuous chromatography systems are also gaining traction as an alternative to batch chromatography, offering higher productivity, reduced buffer consumption and a smaller footprint.

Companies can enhance their chromatography processes by focusing on key areas such as process characterization and understanding, in-line dilution and buffer management, and process modeling and simulation. Understanding biomolecule properties and using multi-mode resins can improve impurity separation, and optimizing buffer management with in-line dilution systems can enhance reproducibility and scalability, while reducing operating costs. Additionally, employing computational modeling tools aids in predicting process performance and troubleshooting, ultimately maximizing efficiency, productivity, and reliability.

Additionally, process intensification strategies, including continuous chromatography and high-throughput chromatography, aim to improve efficiency and sustainability. By integrating sustainability considerations into process design, operation and technology development, the biopharmaceutical industry is actively working towards making chromatography processes more environmentally friendly and socially responsible, contributing to a sustainable future for biopharmaceutical manufacturing.

What lies ahead

The future of chromatography technology in biopharma is poised for significant advancements and transformations. My predictions include a surge in automation and integration fueled by robotics, AI, and real-time monitoring, and more streamlined operations. Automation streamlines processes by minimizing manual intervention and errors, performing tasks, such as column packing, sample loading, and fraction collection, with high precision and consistency, while AI and machine learning could optimize chromatography by analyzing large datasets, predicting optimal process parameters, and facilitating adaptive process control. The result? Faster development, fewer experimental iterations, and improved scalability. Additionally, I expect progress in miniaturization technology and the development of tailored resins that can help improve throughput and selectivity for microfluidic systems and innovative stationary phases. Miniaturization technologies, including microfluidic devices and microscale chromatography columns, offer advantages such as smaller sample volumes, reduced reagent consumption, and faster analysis times – again contributing to sustainability efforts. Real-time monitoring and control capabilities, powered by sensor technology and advanced data analytics, further optimize chromatography processes, ensuring consistent product quality and yield.

Overall, the future of chromatography technology in biopharma holds promise for enhancing efficiency, productivity, and sustainability in downstream processing. Embracing emerging technologies, innovative approaches, and collaborative partnerships will enable the biopharmaceutical industry to overcome current challenges and achieve new levels of excellence in chromatography-based purification of biologics.

Jungmin Oh is Manager, New Product Development, Avantor

Treating the Fentanyl Epidemic

How – and why – we are tackling opioid use disorder with a monoclonal antibody

By Andrew Barrett

The primary challenge we face against the epidemic spread of fentanyl is the long-standing stigmatization of individuals with substance abuse issues. There is limited appreciation that addiction is a chronic, relapsing disease – no different from other chronic conditions – that affects all socioeconomic and demographic stratifications. And it is, at least in part, stigma that has contributed to a lack of investment in this area by healthcare-related venture capitalists.

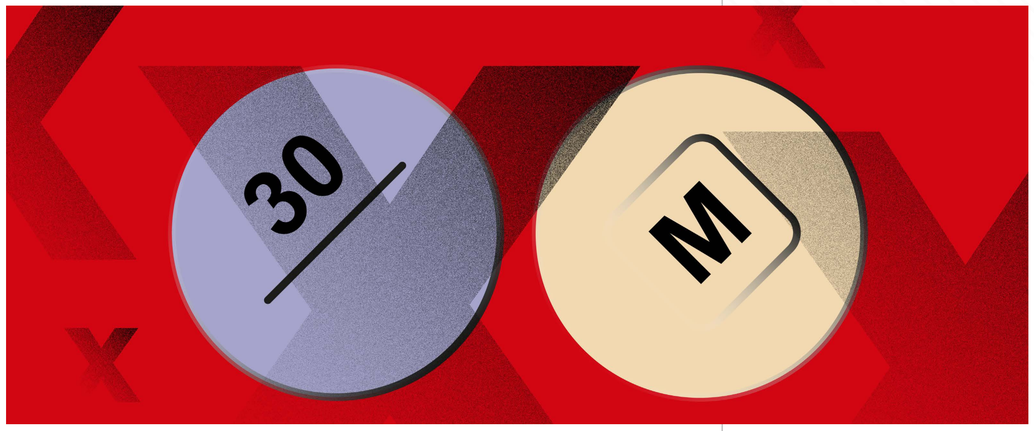
Currently, there are only three molecules approved for the treatment of opioid use disorder (OUD), of which the last novel molecule was approved in 1981. And though these approved treatments do have a long history of safe use, the growing number of overdose-related deaths via fentanyl suggests there is room for improvement. Today, we suffer from a notable lack of therapeutic options; in other words, we are ill-equipped to deal with the scale of the problem.

In the US, fentanyl accounts for over 90 percent of all opioid-related overdose deaths – a rise attributed to fentanyl (and fentanyl analogs) replacing heroin as the primary opioid in the illicit drug supply. What was once an opioid crisis is now very clearly a fentanyl crisis. In most cases, the primary instances of harm, overdose, and fatalities are associated with illicitly produced fentanyl. It is often found in illegal drugs, and the dose varies considerably; even slight alterations in batch-to-batch amounts can result in unpredictable effects (including overdoses or death).

For international crime organizations, fentanyl possesses two notable benefits: it's cost-effective and incredibly potent. To be specific, fentanyl is roughly 10 percent of the cost of heroin and can induce life-threatening respiratory depression in as little as 2-3 minutes, drastically undercutting heroin's 30 minute countdown. Thus, the time window for administering life-saving treatments, such as naloxone (sold as Narcan), is significantly shorter.

There is also evidence to suggest the epidemic could spread beyond US borders. Following a ban on poppy cultivation – the primary source of heroin production – in Afghanistan in 2022, the door has opened for the growth of illicit synthetic opioids. We're now seeing scattered reports of fentanyl-related deaths from countries across the Eastern and Southern hemispheres, as well as in the Baltic states and Brazil (and others) that have certainly intensified this concern. Statistics and reports only tell half the story, and many of us have been touched personally by addiction. Cessation Therapeutics was founded to help change the story. One of our most promising therapeutic candidates – CSX-1004 – has just entered a phase Ia, first-in-human study.

CSX-1004 is a human monoclonal antibody directed against fentanyl – and fentanyl analogs – and works by sequestering fentanyl molecules as they enter the bloodstream, effectively neutralizing them in the blood before they reach the brain, and preventing them from exerting harmful effects. Moreover, because CSX-1004 prevents fentanyl from reaching the brain, CSX-1004



can also block all the effects of fentanyl, including the respiratory depressant effects that lead to life-threatening overdose, as well as the euphoric feeling (or "high") people receive from fentanyl. In a primate model, we demonstrated that a single dose of CSX-1004 blocks the respiratory depressant effects of potentially lethal doses of fentanyl for up to one month.

CSX-1004 is restricted to the bloodstream; thus it does not have intrinsic abuse potential or opioid-related side effects, contrary to other medications for OUD. And because it acts differently than other medications for OUD, CSX-1004 could be used not only as a stand-alone agent, but also in combination with other medications to yield the best outcomes in patients with OUD. We are now working closely with the FDA on further development and recently received Fast Track designation for the molecule.

We are also developing a fixed-dose, subcutaneous formulation of CSX-1004 – known as CSX-1004 SQ – that will provide a formulation to be incorporated into a broad range of healthcare settings. Cessation and its academic collaborator, McLean Hospital/Harvard Medical School, recently received a $14.7 million grant from the National Institute on Drug Abuse (a division of NIH) to spearhead its subsequent progression.

Yet, despite these promising developments, substance abuse is drastically neglected by the private sector. There are still no FDA-approved medications for the treatment of stimulant use disorder, a condition characterized by the problematic use of stimulants, such as cocaine, methamphetamine, and prescription stimulants (amphetamine, methylphenidate). And though there are three approved medications for alcohol use disorder, they have only modest efficacy. Additional treatments are sorely needed. Nicotine use, though not often appreciated as a substance use disorder, also suffers a dearth of pharmaceutical treatment options – and those that are available have modest efficacy and/or tolerability issues.

Though NIDA has been a massive supporter of drug development efforts, we still require much more investment and attention from private investors. In contrast, there are dozens of approved medications for major depression (another chronic CNS disorder), and despite the availability of safe and effective treatment options, there continues to be massive investment in this space. I can only hope that a greater understanding of addiction will eventually reduce the stigma and encourage additional investment in this domain.

Andrew Barrett is Chief Scientific Officer at Cessation Therapeutics

Revenge of the ADCs

Are antibody drug conjugates (ADCs) finally about to live up to the "magic bullet" hype in cancer treatment?

From complex design challenges to issues with linker stability, ADCs have faced many hurdles, but recent promising developments suggest we may be at a turning point. We ask four gurus if the time for ADCs has arrived – and what advancements are paving the way for their success.

The Gurus

Shawn Zhang, Chief Scientific Officer, Ambrx Biopharma
Philipp Spycher, Co-founder and CEO, Araris Biotech
Matt Robinson, Chief Technology Officer, Immunome
Jan Pinkas, Chief Scientific Officer, Pyxis Oncology

Is the term "magic bullets" a fair description of ADCs?

Zhang: This description may not be completely fair because of the difficulties of delivering both safety and efficacy with previous ADC technologies. ADCs are designed to be highly targeted therapies that deliver a potent cytotoxic payload directly to cancer cells. In other words, they are a "targeted chemotherapy."

However, these treatment modalities are only as good as the conjugation method that holds the chemotherapy and the antibody together. For example, ADCs that have unstable conjugation can prematurely release their toxic payload, which can damage healthy tissues, increase drug resistance potential, and deliver inadequate amounts of the cytotoxin to the tumor. Ultimately a "magic bullet" would have sufficiently stable conjugation technology to enable robust on-target delivery of cytotoxin to cancer, with minimal off-target effects to the patient.

Spycher: I actually do think that "magic bullet" is a fair description of ADCs. By combining a highly specific antibody with a powerful anti-cancer drug to target and eradicate tumors, we can potentially eliminate unwanted side effects in other parts of the body. With ADCs, we are attempting to deliver the anti-cancer drug in the most targeted manner possible, thereby avoiding the toxicities we often see with traditional cancer therapeutics. Over the years, there have been some development challenges that have prevented ADCs from reaching that "magic bullet" potential, but the field is in a good place now to start seeing results in practice.

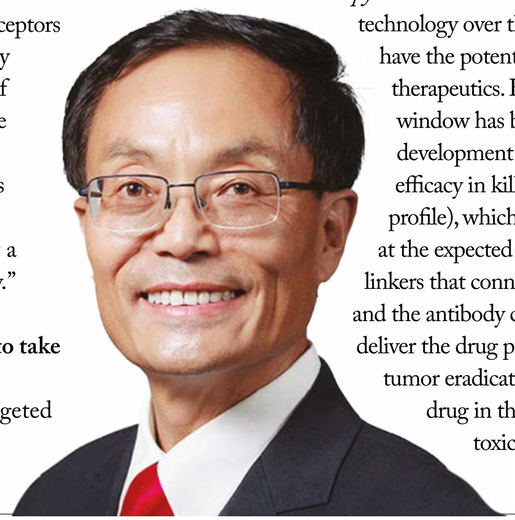
Robinson: Paul Ehrlich's "magic bullet" term has been thrown around for more than a century, and encapsulated his vision that "we need to learn how to aim chemically." In other words, Ehrlich saw an opportunity to target chemotherapeutic agents to receptors present on disease-causing agents rather than healthy tissues, thereby improving the therapeutic window of those drugs. In many ways, the modern ADC can be seen as the realization of his theory.

Pinkas: Based on clinical data from numerous ADCs over the past few decades, I feel that a better description could be that ADCs represent a validated approach for "targeted payload delivery."

Why has it taken so long for the field of ADCs to take off? And are we finally at a turning point?

Zhang: ADCs have been a promising class of targeted cancer therapies for over 20 years, but their development has been challenged by several factors. One key challenge has been the complex nature of ADC design, which requires the combination of a cytotoxic drug, an antibody, a linker, and the conjugation technology that connects the components. Each of these components must be optimized in different ways depending on the cancer types or targets. It can take years to develop an ADC with the desired therapeutic profile.

Despite these challenges, recent advancements in ADC technology have renewed interest in the field. For example, the development of site-specific conjugation technologies has enabled the creation of more precise and stable ADCs, reducing off-target effects and increasing the concentration of the "magic bullet" available to reach tumor sites. In fact, there are a number of promising ADCs now in late-stage clinical trials, as well as plenty more in preclinical development.



Shawn Zhang

Spycher: Tremendous leaps have been made in ADC technology over the last 30 years, and these drugs now have the potential to be highly efficacious cancer therapeutics. However, their limited therapeutic window has been a cause of contention in clinical development (i.e., the balance between clinical efficacy in killing tumor cells and tolerability profile), which explains the lack of broader adoption at the expected pace. Additionally, poorly designed linkers that connect the highly toxic drug payload and the antibody can lead to the inability to efficiently deliver the drug payload to the tumor, thus preventing tumor eradication, or a premature release of the toxic drug in the bloodstream, leading to unwanted toxicities in healthy tissues.

There have also been issues with aggregation of ADCs, which can overall decrease binding of the molecule to the antigen and shorten half-life in the blood. Finally, existing technologies pose challenges of high cost and time to manufacture. With new ADC technologies being developed as well as increased interest in the space, I believe we're at a turning point for the field to take off.

Robinson: Realizing Ehrlich's vision has not been simple for many reasons. ADCs are large, complex molecules whose activities are dictated by a number of different parameters, including 1) selectivity and overall uptake of the antibody into tumor versus normal tissues; 2) stability of the ADC in patients; and 3) potency and mechanism of action of the cytotoxic agent being used. Lots of work has gone into understanding how best to attach cytotoxic agents to the antibody delivery vehicles in a way that provides the necessary improvement in therapeutic window versus free drug. In my opinion, the advances made in those areas over the last 10 years or so are what have led to the resurgence in the ADC field.

Pinkas: The ADC field is entering an exponential phase of growth. As the others explained, the technology has taken time to mature because of its complex design and manufacturing when compared with traditional therapeutics. ADCs use payloads that are upwards of 100 times more potent than traditional chemotherapeutics with highly specific antibodies that target and release the drug at the right location. The entire process is a balancing act between safety and activity. Over time, payloads with distinct mechanisms of action have tuned potency, conjugation strategies have become more precise, and linkers have been developed that are more stable in circulation. The improvement of these technologies may yield a new generation of ADCs that will truly transform the cancer treatment landscape.

What have been the biggest milestones for the ADC industry as a whole over the past two years?

Zhang: Currently, there are 12 ADCs approved by the US FDA, the most recent being Elahere in 2022 for ovarian cancer and Tivdak for cervical cancer in 2021. I think that one of the key milestones is yet to come – using ADCs to treat solid tumor indications. Most approved ADC therapies target liquid cancers, but there is increasing focus now on solid tumors.

Spycher: There have been encouraging investments from big pharma into smaller biotech ADC companies, including major deals between Seagen and Pfizer, as well as GSK and Mersana. Investment confidence in the space is very promising to see.

Robinson: In my opinion, the improvements in linker and conjugation chemistries developed over the last decade have enabled clinical successes that have led to multiple approvals in the space. Perhaps most notably, the approval of Enhertu for the treatment of breast cancer showed how the advances made in the ADC field can be leveraged to substantially improve upon earlier generation therapies and significantly change the standard of care in cancer treatment.

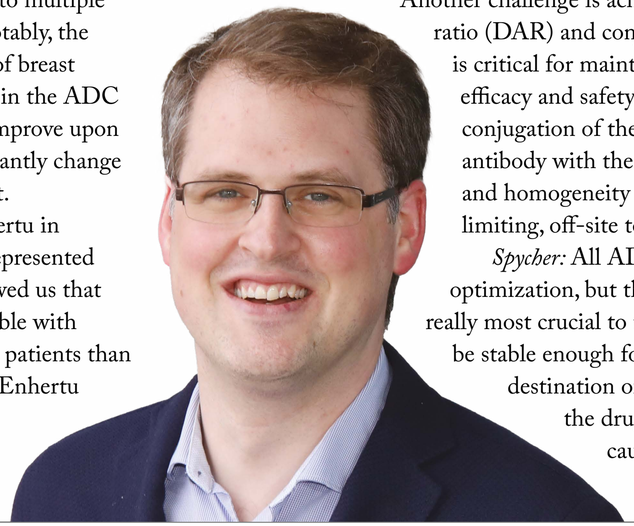
Pinkas: I agree; the approval of Enhertu in people with low expression of HER2 represented a critical moment for the field and showed us that ADCs could go beyond what was possible with traditional therapeutics and reach more patients than previously thought possible. Moreover, Enhertu demonstrates that the potency of the payload is an important component to optimize and that payloads with the highest potency are not always the best.

What are the challenges and biggest discussion points when it comes to optimization?

Zhang: The utility of ADCs is significantly hindered by dose-limiting, off-tumor toxicities. Conjugation plays a critical role in controlling the stability, release rate, and efficacy of the drug payload, and instability within this can lead to premature drug release and toxicity, while linker stability can undermine drug release and efficacy. Therefore, pairing optimal linker design, conjugation chemistry, payload class, and tumor target characteristics is necessary to balance stability and release rate appropriately, and is an ongoing challenge in ADC development.

Another challenge is achieving optimal antibody-drug ratio (DAR) and conjugation site selection. DAR is critical for maintaining the balance between efficacy and safety. By optimizing the site-specific conjugation of the cytotoxic payload to the antibody with the appropriate linker, then stability and homogeneity can be achieved, reducing dose-limiting, off-site toxicities.

Spycher: All ADC aspects require some optimization, but the optimization of the linker is really most crucial to the therapeutic. Linkers must be stable enough for the ADC to make it to the destination of the tumor without releasing the drug payload prematurely and causing off-target toxicities.



Philipp Spycher

Robinson: The optimization process is highly dependent on how each company approaches ADC development. At my company, we believe it's important to keep the focus on novel targets that can enable selective tumor targeting and we have a discovery engine to help with this. Interestingly, our research is revealing novel target classes, such as proteins ectopically (abnormally) expressed on the surface of cancer cells, which we believe are uniquely tumor selective and potentially suitable for development as ADCs.

Pinkas: Conjugation chemistry is a hot topic right now. Historically, the process for assembling the components of an ADC was imprecise, which contributed to many of the toxicity issues. Today, companies are working on new strategies to approach conjugation in a site-specific manner to generate ADCs with more consistent DAR and to improve stability in circulation. Another major topic of conversation is bystander activity. The challenge with targeted therapeutics in oncology is tumor heterogeneity. All of the cancer cells within a tumor may not express the target, and consequently, treatments may be ineffective at completely eliminating cancer. One way around this is to improve bystander activity. Certain payloads, after being cleaved, can migrate to neighboring cells whether or not they express the target and exert their cytotoxic effect. Novel payloads with enhanced bystander activity have the potential to provide a more holistic antitumor strategy and could potentially lead to more durable responses.

What innovation is taking place in linkers?

Zhang: We are working on an expanded genetic code technology platform for incorporation of synthetic amino acids (SAA). Conjugation to the SAA enables the incorporation of an optimized linker-payload at any selected site in the antibody using industry standard cell lines, thus allowing for the generation of engineered precision biologics with site-specific, homogenous, and stable conjugation.

I'm also seeing the industry exploring a lot of new conjugation technologies, such as enzyme-based or sugar-based chemistries.

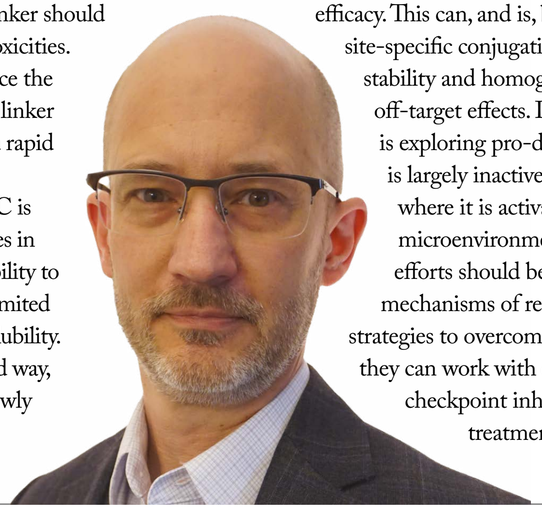
Spycher: It's been shown that in addition to stability of the linker being crucial for ADC success, linkers also play a role in clearance of the ADC. Some of the first innovations used labile and hydrophobic linkers resulting in poor efficacies, pharmacokinetic ADC profiles, and ultimately limited tolerabilities. At my company, we're working to create hydrophilic and highly stable linkers that allow for straightforward conjugation of the payload drugs, taking off the shelf antibodies and using them in our ADCs. We're able to retain the biophysical properties of the antibody thanks to the biochemical nature of the linker, which enables us to maximize exposure of the toxic drug to the tumor with only minimal toxicities. In addition, we believe that the release of the payload from the linker should be highly controlled in order to avoid excessive toxicities. This is because for many conventional linkers, once the ADC gets internalizated in whatever tissues, the linker will be cleaved instantly which will then lead to a rapid payload release causing unwanted toxicities.

Robinson: History has taught us that each ADC is bespoke. From my perspective, the biggest advances in linkers are those that provide scientists with the ability to tailor attributes of the therapy, including but not limited to DAR, stability, site-specific conjugation, and solubility. Each of these can then be applied, in a coordinated way, to evaluate their contributions to the efficacy of newly developed ADCs.

Pinkas: Numerous advances have been made in linker chemistry to improve stability in circulation while maintaining efficient release in the tumor. Clinical data with ADCs comprising linker formats with a range of stability in circulation suggest that payload release contributes to toxicity. The concept of "cleavable" and "non-cleavable" linkers is outdated, and we should describe linkers based on their stability in circulation and the properties of the payload upon release in the tumor.

Where do you think the priorities should lie when it comes to furthering the ADC field?

Zhang: Based on current research and trends in the field, there are several priorities that can be considered, the biggest being improving the safety profile of ADCs. Approved ADCs such as Enhertu have shown promising results, but there is still a lot of room for improvement in terms of minimizing toxicity while maximizing efficacy. This can, and is, being done by further advancing site-specific conjugation technologies to improve the stability and homogeneity of ADCs and minimize off-target effects. In addition, some early research is exploring pro-drug approaches, where an ADC is largely inactive until it enters the tumor site, where it is activated by tumor proteases or other microenvironmental factors. I also believe research efforts should be directed towards understanding the mechanisms of resistance to ADCs and developing strategies to overcome them as well as understanding how they can work with other therapies in combination (i.e., checkpoint inhibitors, to produce the most effective treatment regimens).



Matt Robinson

Spycher: Further enhancing linker technologies that allow for fine tuning of stability and conjugation of the payload to the antibody should be at the forefront of ADC development. A strong linker foundation sets up the ADC for success, but I feel that linkers have been greatly undervalued in the ADC space. For example, depending on the amino acid sequence used for the linker, potential dose-limiting toxicities can be much better controlled. In my view, there is no such story as a "one-size fits all linker." For each antibody and payload combination, linker optimization is necessary to maximize payload delivery to the tumor. Thus, linker performance sets the stage for the efficacy, safety, and tolerability of ADC therapeutics.

An additional consideration for the development of ADCs is the beneficial impact of high drug-to-antibody ratio (DAR). As we advance ADCs, we may find that high DARs are not necessary when using a low potency warhead. Ratios of 4 or less may be beneficial, and allow for high dosing and achieve high tumor penetration.

Robinson: While I believe future advances in linker, drug, and conjugation chemistry will continue to progress the ADC field, I also believe that a better understanding of the target landscape is going to be critical to fully realize Ehrlich's vision of the "magic bullet." Ehrlich postulated the need for receptors that are selective for disease versus normal tissues. The currently approved ADCs are focused on a small subset of targets, and hence small subset of cancers, with significant room to expand. The data we are generating at my company – through the interrogation of patients' antibody responses against their disease – has uncovered unique areas of biology that highlight novel target classes with the potential to provide increased tumor selectivity as compared to current targets. The better we understand those target classes, the more we will be able to select the right targets with potential to have the greatest benefit for patients, hopefully across multiple cancers.

Pinkas: While innovation is needed on all fronts, the biggest impact will come from improvements in conjugation strategies, since this can broadly translate to improvements across ADCs. The second priority is payloads. Not all payloads are created equally, and each employs a different mechanism of action, which may be more effective against certain types of cancers. Excitingly, newer payloads have been shown to induce immunogenic cell death, meaning that the drug kills the cancer cell and primes the immune system. This has major implications, especially in a combination treatment setting with other immuno-oncology drugs like checkpoint inhibitors.

Please make a bold prediction for the coming years…

Zhang: We will one day see ADCs replace standard chemotherapy treatment and become the standard-of-care for cancer treatment. With the approval of newer and more effective ADCs, and with the ongoing development of next-generation ADCs with improved targeting, potency, and safety profiles, the field is poised for significant growth. Additionally, as personalized medicine becomes more tailored as we gain a wealth of individualized data, ADCs with the ability to target specific cancer subtypes could become an increasingly important tool in the fight against cancer. This will include the identification and validation of new cancer targets, which would be invaluable for our field, industry, and most importantly, for patients with solid tumors who have long awaited consistently reliable treatment options.

Spycher: When looking at how ADCs have already altered the treatment paradigm for certain cancer indications, essentially re-defining how patients are treated and the impact on their quality of life, it seems to me that ADCs are primed to play key roles for many cancer indications. Eventually, they may replace conventional chemotherapy and live up to their initial promise of being "magic bullets."

Robinson: Leveraging modalities, such as ADCs, may provide a more linear clinical translation in drug development. I expect that better understanding of cancer biology and the expression of targets on the surface of solid tumor cells, specifically in the context of the tumor microenvironment, will expand the landscape of tumor targets addressable by ADCs, leading to multiple clinical milestones and additional approvals in the coming years.

Pinkas: ADCs will become first-line treatments for many different indications. In oncology, we've seen the rise of many new treatment modalities, but we don't often see drugs breaking into first-line treatments. As the industry becomes more sophisticated in the design and development of ADCs, we'll start to see them become more prevalent first-line options.

Further down the road, I could envision ADCs being used outside of oncology. The beauty of this technology is that it's really a delivery system for highly potent small-molecule drugs. In fact, we could potentially apply this strategy to deliver different agents that, for example, suppress the activity of cells responsible for autoimmune disease.

Jan Pinkas

Unlocking the Future of Biomanufacturing

How a continuous manufacturing approach is key to cutting the costs of biologics production

Over the past two decades, biologics have emerged as game-changing therapies for numerous health conditions, yet many patients globally cannot access these life-saving medications. One of the biggest factors limiting access to biotherapeutics is the high manufacturing costs.

Fed-batch processes are becoming increasingly viewed as a limiting factor in biologics production, bringing process inefficiencies, limited scalability, and operational risks that hinder production and contribute to high costs. As clinical indications for biotherapeutics continue to expand, the demand for more productive, agile, scalable, and cost-effective manufacturing processes has never been greater.

Just – Evotec Biologics is leading the charge in addressing these challenges through the development of a transformative continuous manufacturing platform that prioritizes process agility, risk reduction, and cost-efficiency. This forward-thinking approach offers sponsors a chance to break the biomanufacturing bottlenecks that have, until now, constrained access to critical biologics worldwide.

An agile approach to biomanufacturing

Continuous manufacturing marks a significant departure from traditional fed-batch processes. Unlike fed-batch, where production occurs in separate sequential steps with necessary downtime between cycles, continuous manufacturing units operate in a steady state, allowing for uninterrupted production.

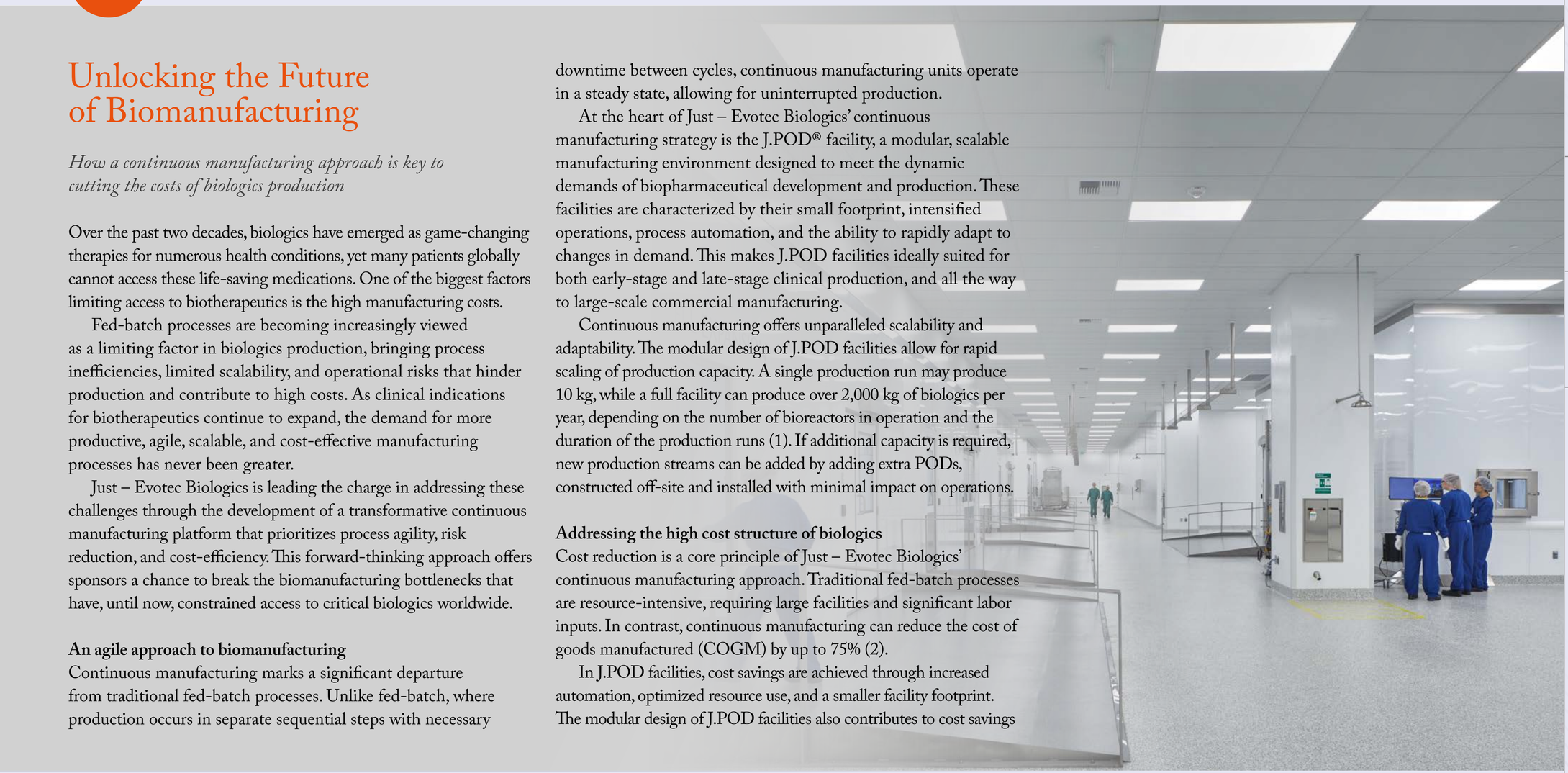
At the heart of Just – Evotec Biologics' continuous manufacturing strategy is the J.POD® facility, a modular, scalable manufacturing environment designed to meet the dynamic demands of biopharmaceutical development and production. These facilities are characterized by their small footprint, intensified operations, process automation, and the ability to rapidly adapt to changes in demand. This makes J.POD facilities ideally suited for both early-stage and late-stage clinical production, and all the way to large-scale commercial manufacturing.

Continuous manufacturing offers unparalleled scalability and adaptability. The modular design of J.POD facilities allow for rapid scaling of production capacity. A single production run may produce 10 kg, while a full facility can produce over 2,000 kg of biologics per year, depending on the number of bioreactors in operation and the duration of the production runs (1). If additional capacity is required, new production streams can be added by adding extra PODs, constructed off-site and installed with minimal impact on operations.

Addressing the high cost structure of biologics

Cost reduction is a core principle of Just – Evotec Biologics' continuous manufacturing approach. Traditional fed-batch processes are resource-intensive, requiring large facilities and significant labor inputs. In contrast, continuous manufacturing can reduce the cost of goods manufactured (COGM) by up to 75% (2).

In J.POD facilities, cost savings are achieved through increased automation, optimized resource use, and a smaller facility footprint. The modular design of J.POD facilities also contributes to cost savings



Credit: Benjamin Benschneider

J.CHO™ High Expression System

Tech partnership with Sandoz

Step into the era of continuous biomanufacturing

by allowing for incremental expansion as needed, rather than requiring significant upfront investments in large manufacturing facilities.

Another way costs can be significantly reduced is by using the J.CHO™ High Expression System, a proprietary CHO-K1 cell line that maximizes the yields, quality, scalability, and cost-effectiveness of the continuous manufacturing process.

Mitigating process risks

By adopting continuous manufacturing, sponsors can mitigate a number of critical risks associated with fed-batch systems. Continuous bioprocessing minimizes hold phases between process steps, minimizing the opportunity for contamination, while process automation reduces the incidence of human error.

This biomanufacturing approach ensures that product quality attributes are more consistent, reducing the likelihood of batch failures and the need for costly rework. Moreover, J.POD facilities enable advanced process monitoring and optimal control over process conditions, further minimizing risk through process and product consistency.

By establishing facilities in multiple regions, including North America and Europe, the continuous manufacturing platform also mitigates geopolitical risks and ensures supply chain stability. Each J.POD facility is standardized, meaning processes can be seamlessly transferred between a growing global network of facilities.

Leading the charge in Europe

Just – Evotec Biologics recently unveiled their latest cGMP J.POD manufacturing site in Toulouse, France, representing the only end-to-end fully continuous manufacturing facility in Europe. This cutting-edge facility will support European customers, including Sandoz, with clinical and commercial biomanufacturing. Customers can also benefit from end-to-end product and process development, with capabilities including cell line development, upstream and downstream process development, and formulation development.

From batches to brilliance

For those looking to stay ahead in the competitive biopharmaceutical landscape, partnering with Just – Evotec Biologics offers the opportunity to leverage over a decade of expertise in continuous manufacturing, in combination with innovative technology and facilities. This ultimately reduces costs and risks, while optimizing product quality and supply.

The CDMO supports partners through each and every step of the development and manufacturing process, from early-clinical stages to commercial supply. In addition to traditional CDMO fee-for-service approaches, flexible, integrated programs can be developed, along with technology out-licensing opportunities.

Step into the era of continuous biomanufacturing

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J.CHO™ High Expression System

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mAbs: Not so Sweet

How fucosylation-deficient CHO cell hosts can help enhance the potency of monoclonal antibody-based biotherapeutics

By Neha Mishra and Jesus Zurdo

Early on, one of the central challenges in mAb production was low product titer – but this has since been overcome by advances in cell line development and substantial improvements in cell-specific productivity, and further driven by broader progress in industrial bioproduction technology. Bioprocess optimization, generally achieved via optimization of media and culturing conditions (temperature, speed, etc.), has led to significant improvements in product titers and performance of the host cells. Meanwhile, the ever-increasing demand to develop and manufacture mAbs has led to heavy investment in R&D programs focusing on product quality and consistency.

Approval of biotherapeutics for human use requires the definition and control of a number of critical quality attributes (CQAs) which are key to performance and safety. For mAbs, the presence and type of post-translational modifications (PTMs), such as glycosylation, is a good example (1).. Glycosylation is an enzymatic process involving the addition of oligosaccharide structures to specific amino acid sites of polypeptides to form glycoproteins. This non-template based process occurs within the endoplasmic reticulum (ER) and Golgi as the protein transits through the cell before secretion or translocation. There are many forms of glycosylation, but the two most common types are N- and O-linked glycosylation:

- In N-linked glycosylation, oligosaccharides are attached to the amide nitrogen of an asparagine (Asn) residue in a consensus sequence Asn-X-Ser/Thr where X is any amino acid except proline.
- In O-linked glycosylation, oligosaccharides are attached to the oxygen atom of hydroxyl groups of amino acids such as serine (Ser), threonine (Thr) or tyrosine (Tyr).

The glycan core structure (see Figure 1) presented by antibodies contains N-acetylglucosamine (GlcNAc) and mannose upon which other sugar residues, such as galactose, sialic acid and fucose, are added.

Why is glycosylation so important in proteins?

Approximately 70 percent of mammalian proteins are glycoproteins with N-linked glycans, which often confer specific properties to the polypeptide chain. Variation in N-glycosylation of therapeutics can have a significant impact in protein folding, stability, pharmacokinetics, immunogenicity, or even mode of action (2, 3). This impact is particularly relevant for mAbs, where variability in the N-glycan structures present in the CH2 domain determines, amongst other things, cell-mediated responses, including antibody-dependent cell cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC).

Given the influence of specific glycans on the therapeutic effect of biologics, the control of glycosylation profiles in biopharmaceuticals, is a highly important topic.

ADCC responses are mediated by the FcγRIIIa (CD16) receptor expressed primarily by natural killer (NK) cells (also known as effector cells). Antibodies recognizing specific ligands on a "target-cell" surface can activate NK cells through the interaction between the Fc region of the antibody and the FcγRIIIa receptor of an NK cell, resulting in release of cytotoxic agents that ultimately eliminate the target cell (see Figure 2). The magnitude of the ADCC response is dependent on the affinity between the FcγRIIIa receptor and antibodies (4). Structural studies have revealed that the presence of fucose on the core glycan structure on IgG1-Fc reduces binding affinity of the IgG1 to FcγRIIIa receptors (5). Therefore, the removal of core fucose in glycan structures of antibodies – known as afucoslylation – is a particularly important strategy in oncology therapeutics.

Advantages of afucosylated antibodies include:

- Effective ADCC responses against tumors exhibiting low antigen-expressing levels. This can be relevant for cancer therapeutics, such as Rituximab, which has been shown to be less effective against lymphomas with reduced CD20 expression (6). The ability of afucosylated mAbs to elicit ADCC responses against cells with low antigen expression levels opens the door to more effective therapeutic approaches against currently unsuitable oncology targets.
- Reduced competition from serum IgGs in binding (and activating) FcγRIIIa receptors. Evidence in clinical settings has shown that therapeutic antibodies can be inhibited by circulating IgG competing for FcγRIIIa receptor binding (7). Higher levels of therapeutic antibodies are therefore required to overcome this competition, which can introduce complications and undesirable side-effects. The use of afucosylated antibodies can reduce such competition by increasing the binding affinity to FcγRIIIa receptors.

By addressing these two factors, afucosylated antibodies could have a significant impact in increasing the potency of biopharmaceuticals, expanding their therapeutic window, and potentially reducing undesirable side-effects and complications associated with treatment, due to the lower doses required to elicit a physiological effect.

The use of glycoengineered mAbs is not restricted to oncology therapies. Complement-dependent cytotoxicity (CDC) is also affected by the glycosylation pattern; antibodies exhibiting low or no galactose and high mannose show a decreased binding to complement component 1q (C1q) complex, leading to a reduced CDC response. Additionally, highly sialylated antibodies can mediate anti-inflammatory responses in autoimmune diseases (8).

Given the importance of glycosylation on effector functions that are mediated by therapeutic antibodies and Fc-fusion biotherapeutics, host cell lines used to express such products can be engineered to produce selective glycoforms that can, in turn, modulate their specific biological activity.

The right tool for the job…

Chinese hamster ovary (CHO) cells have been used for biologics production since the approval of t-PA in 1987. CHO cells can produce human-like PTMs and are robust systems capable of adapting efficiently to different culture conditions, including

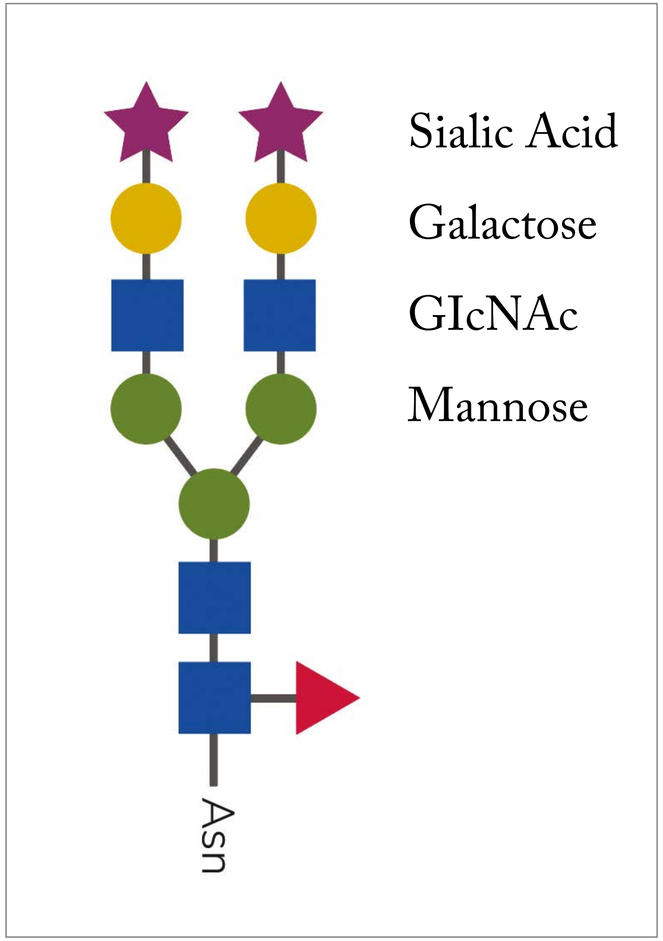

Figure 1. Glycan core structure

serum-free media. Importantly, CHO cells are less prone to being infected by human viruses. Recent advances in bioprocess engineering have dramatically increased the performance of these cells and the yields typically obtained in bioproduction (9).

CHO cells usually produce high proportions of fucosylated mAbs, impacting the biological activity of antibody therapeutics they express. Equally, as stated above, other glycan modifications can drastically influence the effector function of mAbs. Therefore, there is great potential in the modification of the glycosylation pathways of CHO cells to generate therapeutics with improved properties. For this, the use of next-generation genome editing tools can offer an effective tool to engineer expression hosts able to produce therapeutics with specific characteristics (10).

There has been a growing interest in controlling the glycan composition of therapeutic proteins, particularly to generate more efficacious therapies by eliminating the fucose content of mAbs. To enrich the proportion of afucosylated antibodies in the final product, several strategies have been explored: (i) control of cell host (CHO primarily) metabolism during cell culture conditions, (ii) inhibitors targeting fucosyltransferase or other fucosylation enzymes, (iii) expression of enzymes to deviate metabolism, reducing available fucose in the cells, and (iv) use of RNAi to repress or reduce transcription of key fucosylation enzymes, amongst others.

However, glycan composition is highly sensitive to external conditions, product, and overall behaviour of cells in culture. Consequently, this creates a problem for developers on two fronts: i) most of these technologies make it virtually impossible to generate therapeutic preparations with 0 percent or 100 percent of their molecules containing a given glycan composition (8), and ii) batch-to-batch variability observed in bioproduction is intrinsically inherent to the nature of the cell culture control systems – and can have significant consequences in drug potency and safety. The latter is particularly acute because potency cannot be simply traced to dose anymore and batch-to-batch variations in glycan composition can have a substantial impact in drug potency. This places additional stresses on manufacturing and quality control that are very difficult to address.

Therefore, there is great potential in the modification of the glycosylation pathways of CHO cells to generate therapeutics with improved properties. In this regard, next-generation genome editing tools can help engineer expression hosts able to produce therapeutics with specific characteristics (10).

When it comes to fucose, one obvious answer lies in engineering host variants that lack the ability to incorporate a fucose molecule in the glycan structure (11). In these types of systems, it is possible to use a functional knockout of a fucosyltransferase gene to inactivate the fucosylation pathway in the cells. Antibodies expressed from these cell lines contain glycans that are devoid of the core fucose as shown by glycan analysis, where 0 percent of fucose is detected. In comparison, mAbs produced from the wild-type parental cell line contain up to around 90 percent of fucosylated glycans (see Figure 3). Afucosylated model antibodies exhibit markedly higher efficacy in eliciting an ADCC response than their fucosylated counterparts when faced with target cells with low antigen-expressing cells and in the presence of NK cells with FcγRIIIa receptor polymorphisms that are known to decrease ADCC functionality (12).

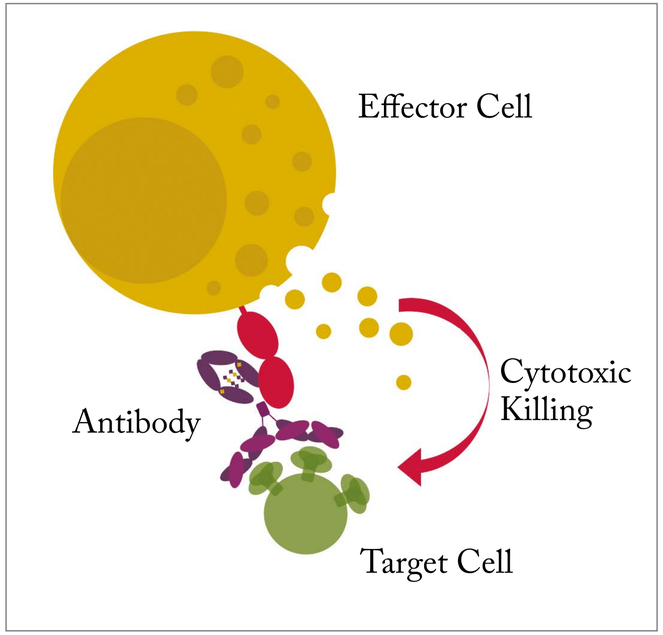

Figure 2. ADCC mediated by effector cells: ADCC response on a target cell via CD16 receptor on an effector cell, triggered with the help of a mAb (figure adapted).

Two glycoengineered mAbs lacking fucose, anti-CCR4 mogamulizumab and anti-CD20 obinutuzumab, have been approved for therapeutic use in 2012 and 2013, respectively (both produced in genetically modified CHO cells). Many more glycoengineered mAbs lacking fucosylation are currently in development in areas as diverse as oncology and infectious diseases (8).

Understanding glycobiology

As outlined above, glycan composition is well known to modulate the biological activities of antibodies in our bodies – from regulating half-life to eliciting ADCC or CDC immune responses. Typically, these functions are mediated via endogenous Fc receptors present in different cell types and tissues and influenced by their relative affinity for different Fc architectures (including different amino-acid and sugar compositions).

Glycobiology is, therefore, emerging as an important discipline in the design of more effective biotherapeutics, particularly by modulating effector function in the case of IgG molecules. As we've also highlighted, gene editing technologies can be used to engineer host cell lines able to produce afucosylated therapeutic antibodies to enhance ADCC response; indeed, antibodies lacking fucose in their Fc glycan show up to 50-fold increased binding affinity to FcγIIIa receptors of NK cells mediating effector ADCC responses (12, 13). The absence of fucose residue also compensates for the differences in effector function activities across human populations with different polymorphisms in position 158 of the FcγIIIa receptor. More broadly, afucosylated antibodies have shown improved patient responses and outcomes, irrespective of the amino acid present at such a position (13). And this adaptive immune response has much wider applications beyond the development of treatments for oncology, opening the door to applications in a wide range of conditions where better control over ADCC effector function activity is desirable.

The development of antibodies with enhanced ADCC activity has also been increasingly explored in the treatment of infectious diseases, particularly viral infections; there is a growing body of evidence supporting the use of cytotoxic mechanisms of action to control the spread of infection within patients affected by a given virus. This approach has been successfully assessed against a number of different infections, including Ebola virus, human immunodeficiency virus (HIV), respiratory syncytial virus (RSV), and influenza (14).

In short, genetically modified CHO cells can be used to produce afucosylated antibodies with enhanced ADCC activity, which can drive the development of more effective treatments in oncology, infectious diseases, and autoimmune disorders, while offering greater control over product quality and potency.

Neha Mishra is Senior Scientist Bioproduction

Jesus Zurdo is Global Head Cell, Gene Therapy & Bioproduction at PerkinElmer's Horizon Discovery

See references online

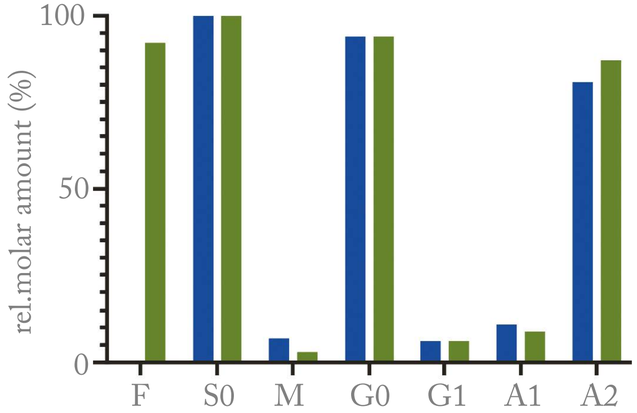

Figure 3. N-Glycan structures detected on trastuzumab (TTZ) control mAb expressed in a wild- type and glycoengineered CHO cell line detected by HIL-IC-HPLC. TTZ produced in the glycoengineered cell line shows complete removal of fucose from the glycoprotein (data from PerkinElmer's Horizon Discovery).

Link
Online Article

Biologically Driven

Academia has its appeal for many; for others the real challenges are in industry. Here, IO Biotech CEO Mai-Britt Zocca discusses her own motivations

A medicine making career often begins in academia. So what drives an academic into industry? The same thing that drew them towards academia, according to IO Biotech founder and CEO Mai-Britt Zocca – curiosity and a desire to use one's intellect to help improve quality of life for patients. And once established in industry, it's the challenges (and overcoming them) that maintains the appeal.

How did you come to focus on translational science and clinical immuno-oncology?

It dates back to my PhD days at the US National Cancer Institute, where I studied patient reactions to cancer vaccines. Seeing how the immune system worked in patients and how we could drive T cell responses was really exciting – and that passion has never faded. We have come a long way since then, and we are now seeing how science in the immuno-oncology field is delivering value in clinical settings. In a translational setting, we are learning how the biology works in patients and how we can now better adapt trial designs to see improved outcomes. It's truly exciting to be in this field and be a part of the developments that we are seeing.

"Therapeutic cancer vaccines is a field that hasn't really proven itself yet, but there is a big wave coming, with multiple players working to drive the area forward."

Why make the shift to industry?
Immunology has always drawn my attention. When I started to understand how we were able to drive a specific response from cytotoxic T cells towards cancer cells and translate that to patients, I decided to join the NIH. As we began to understand how we could launch viable products, I became interested in the biotech space and commercial drug development.

What does a biotech offer that perhaps big pharmaceutical players cannot?
Biotechs tend to have very short decision paths with few layers, which means things can move quickly. IO Biotech was founded in 2014 and just 10 years into the journey we are in a pivotal phase III trial; I think that's quite fast, even in biotech settings. And that is what is attracting more seasoned CEOs into the biotech field. What we are really in it for is the innovative medicines that can drive changes in the landscape – not only for a small patient group but for new treatment strategies that will have an impact in broader settings.

Therapeutic cancer vaccines is a field that hasn't really proven itself yet, but there is a big wave coming, with multiple players working to drive the area forward.

How important is collaboration and surrounding yourself with the right partners and players?
It is really important – and not only for where we are today, but also where we were some years ago – and where we hope to be years from now. If you look at our pipeline, we are working across several indications and getting drugs delivered by Merck Sharp & Dohme via a drug supply agreement. Take our phase III trial as an example; it's a big trial involving several hundred patients receiving pembrolizumab or anti-PD 1 antibodies. This is very costly, so for us it's a valuable collaboration.

We also have collaborations with some of the large cancer institutions in Europe and the US. We have recently launched an investigator-initiated trial with Memorial Sloan Kettering that is now enrolling patients in a metastatic melanoma trial so we can begin testing our cancer vaccine therapy in a new combination alongside our lead program.

What would you be doing if you weren't leading a business like IO Biotech?
I always identify myself as a founder of companies, with IO Biotech being the fifth company that I have participated in founding. I'm very excited about the energy that comes from founding companies. But if I wasn't here, I would probably be in a hospital or an academic setting, where I would look more into understanding the exciting ways that biology and immunology can work together to help patients.

Give us a bold prediction for how the sector might look in 5-10 years time…
I see a future where we will have approvals of several new medicines that will change and improve the outcome for many patients in need. I'm not only thinking about oncology; there are many interesting developments in neuroimmunology where we haven't seen many new drugs in many years.