Volume 2 Issue 2

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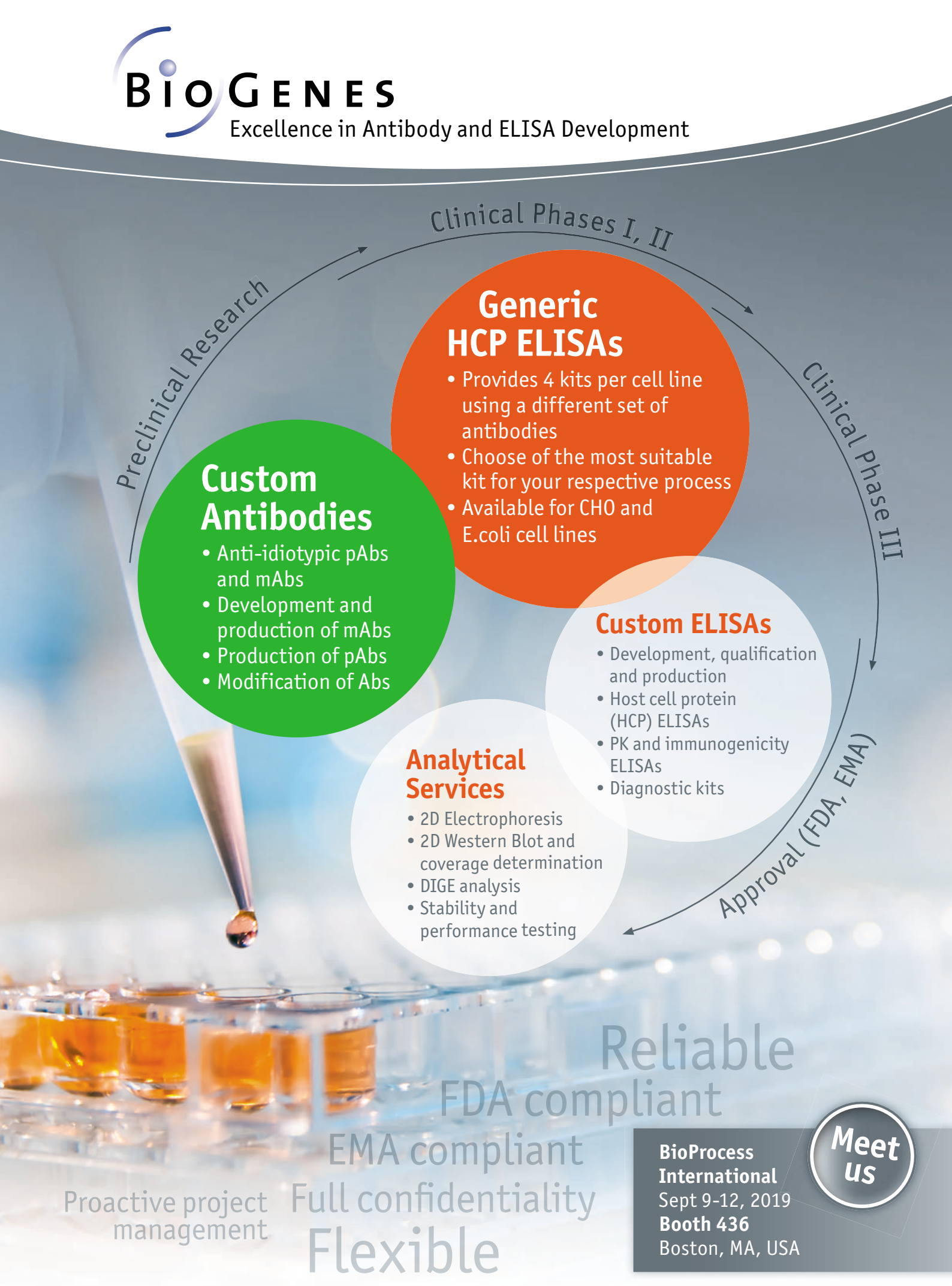

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PHARMA PUBLICATIONS
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Foreword

The major multinational pharmaceutical companies, which were once considered traditional pharma companies, are now shifting their presence into biopharma on an unprecedented scale. However, with this comes growing pains. Looking at the industry growth and the complexities of biopharma development, it is no wonder that transformational developments are required to better equip laboratories and operations in biopharma.

The pharmaceutical industry is going through the revolutionary phase of moving from small molecule drugs to large molecule biological drugs. Biopharmaceutical sales alone have reached $218 billion (USD) in 2017, with a Compound Annual Growth Rate (CAGR) of more than 8%. This constitutes around 20% of total pharmaceutical sales and doubles the growth rate of conventional pharmaceutical industry. Biopharmaceutical drugs target disease pathways and provide superior efficacy and safety. Biologics also made the previously untreatable disease treatable. These key features have made the biologics widely accepted by the public, driving the demand for heavy investment in biopharma R&D. Biotechnology patents are increasing yearly at 25% growth rate. Currently, over a third of all new drugs in clinical trials are biologics and the success rate of biologics is higher than small molecule drugs. In a recent survey of global pharma lab managers, it is no surprise that 56% stated obtaining higher throughput and productivity in their laboratories is their primary goal, and 53% are looking to improve system efficiency.

The advancement of biotechnology opened the door to designing new types of therapeutic drugs to fight disease in a sophisticated manner. Biopharmaceutical products are diverse, including monoclonal antibodies, bi-specific antibodies, antibody-drug conjugates, fusion proteins, recombinant proteins, growth factors, hormones, synthetic immunomodulators, recombinant enzymes, vaccines, synthetic peptides, and oligonucleotides. Monoclonal antibodies (mAbs) and its derivatives have emerged as the largest group of biopharmaceuticals on the market.

I wish to highlight some key articles of major interest in the industry.

Our Research & Innovation section contains an article by Dr Chris Doherty, who talks about the significant changes taking place in R&D and healthcare globally due to Brexit and drug discovery becoming more open and collaborative. In his opinion, this is a good time for exploring how the UK's strengths can be built upon.

Among other interesting articles in our Clinical Research section, we have a piece by Nitish Mittal and Kanika Gupta at Everest Group who discuss how the life sciences industry is undergoing a fundamental change in its business model. They explain why pharma and research companies are turning to digital technologies to transform clinical development.

Within our Manufacturing / Technology Platforms section, an article by Guillaume Tremintin et al of Bruker Daltonics describes the importance of drug safety and purity. The authors write that there is an ever-increasing requirement for analytical methods with rigorous process-related impurity testing. These monitor the contaminants during manufacture and prior to product release.

Patient safety will always be the top priority of every clinical trial. Within the Regulatory / Quality Compliance section, we have a piece by Brian Keesee at PCI, who explains how the industry is adapting better solutions to effectively handle pure, safe and effective patient safety.

I hope you all enjoy this issue of IBI, and my team and I look forward to bringing more exciting articles in the Autumn issue.

Virginia Toteva, Editorial Manager

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Driving Sci-tech Growth in the UK Regions

Sports psychologists place a great deal of faith in the value of confidence. To make the most of your abilities you need an appreciation of your own worth and abilities – the expectation that you will succeed. Much the same is true in industry. Pushing any business or sector forward requires self-belief and faith in the proposition.

The UK has a long-standing reputation as a world leader in the research and development of new medicines and for operating a high quality life sciences industry. With the Brexit era upon us and significant changes taking place in R&D and healthcare globally, including drug discovery becoming more open and collaborative, it's a good time to explore how we can build on the UK's strengths.

When it launched the Industrial Strategy in 2017, the UK government was keen to stress that we are an open, enterprising economy, built on invention, innovation and competition. Our universities and research institutions are among the best in the world. We have a deserved reputation for being a dependable and confident place to do business, with high standards, respected institutions, and the reliable rule of law.

We are also a crossing point for the world because of our geographic position and the English language.

Science and technology is a priority area and recognised as one of the most effective means of achieving the desired goal of a highly skilled, high value economy. Life science is key here – and is the subject of not one, but two sector deals. The first was about joint commitments between the government and the sector to invest in the life sciences landscape. The second, launched at the end of 2018, sets out significant actions to transform the prevention, diagnosis and treatment of chronic diseases by 2030.

It's encouraging to see the priority given to our industry, but the UK has a deep science base. In the blueprints for how we take the sector forward, it's important that policy-makers do not overlook where the seeds of enterprise are growing.

Universities are at the core of our ability as a nation to nurture the key ingredients of innovation: acquiring new knowledge, comparing ideas, seeking solutions, fostering inventiveness. The UK's 24 leading research universities are represented by the Russell Group and collectively inject nearly £87 billion into the national economy every year. Some 17 of the 24 universities are based outside of the 'Golden Triangle' of London, Oxford and Cambridge. They are fundamental to how we develop the life science economy, collectively providing the framework on which the UK can further develop a high value, highly-skilled economy.

The imperative to do more for the regions is reflected in a wider sense by research conducted by the accountancy firm EY. In its UK Regional Economic Forecast, published at the end of 2017 and the most recent data available, it found that there has been little progress on geographical rebalancing of the UK's economy in the last three years. Furthermore, the economic divide between the north and south of the UK was forecast to expand over the next three years, albeit at a slower rate than previously. London and the south east will continue to outperform all other UK regions through to 2020 with gross value added (GVA) growth per year averaging 2.2% and 2.0% respectively, compared to 1.8% for the wider UK.

The second life science sector deal from the end of 2018 does contain some £1.6bn of planned industry investment in the north over the next five years. The Northern Health Science Alliance (NHSA), which creates collaborative research partnerships across the north's leading universities, research-intensive NHS trusts and four academic health science networks, has in particular welcomed the package. It points out that the Northern Powerhouse is home to 21% of the total UK life science sector workforce, and has grown by more than 9% to over 50,000 employees since 2012. Latest figures for 2017 show the value of this sector in the north to be over £13.6 billion.

The sector deals do not exist in isolation. In 2015, the government announced science and innovation audits (SIAs) in order to foster a new approach to regional economic development. We are seeing some progress here. Now in their third wave, SIAs are helping to galvanise sci-tech related enterprise across the



country. But the volume still needs to go up on the message to central government: the regions are not looking for hand-outs, but rather recognition of what can be achieved by building on the intellectual and entrepreneurial base around the country.

Funding is, of course, a critical component of success. The industrial strategy set out the laudable objective of the government working with industry to boost total spending on research and development to 2.4% of GDP by 2027. The context here is worth keeping in mind. According to World Bank figures, Korea (4.2%), Japan (3.3%), the United States (2.8%), Germany (2.8%), Switzerland (2.4%), France (2.2%), and China (2.07%) are already making an appreciably bigger commitment to R&D.

This compares to the EU average of 2.03%, and puts the UK 22nd on the list of OECD countries, behind the likes of the Czech Republic, Iceland and Slovenia. Why does this matter? R&D is the key driver of economic growth, and of productivity growth. The Office of National Statistics has itself observed that "overall R&D intensity in the UK has been fairly flat throughout the 21st century, at or around 1.6–1.7%." It says that by focusing on civil (non-defence related) R&D, in 2016 some 53% of all R&D performed in the UK was funded by businesses, 8% by higher education institutions or funding councils, and 17% by government, including the Research Councils.

Another key issue is technology transfer. According to research conducted by Professor David Gann and Professor Nick Jennings at Imperial College London, the best UK universities compare favourably internationally. Oxford, Cambridge, Imperial, UCL, Manchester and Edinburgh regularly benchmark their performance in technology transfer. This includes the number of invention disclosures per £100 million investment in research, where they calculate that the UK (74) outperforms the US (58). In patent productivity, the UK slightly outperforms the US, and in IP income, it is catching up: the US achieves around 4% of research resource, the UK 3%.

The professors ask quite simply how can the flow of ideas be accelerated – and then point the way to the answer. They call for substantial new support for the innovation clusters in major cities that are growing around research-intensive universities. In my view there's in fact a great deal of consensus around this point – innovation clusters/districts are one of the best routes to meeting the objective of the public and private sectors investing 2.4% of GDP in R&D by 2027.

The potential in cities like Manchester, Liverpool, Leeds, Birmingham and elsewhere – all cities with fine universities – is palpable and was one of the drivers behind the creation of Bruntwood SciTech in October 2018. The new venture was formed by leading property company Bruntwood and Legal & General Capital, one of Europe's major institutional investors.

The business has nailed its colours to the mast of the regions, starting life as the custodian of the UK's largest portfolio of science and technology assets. It includes more than 500 science and technology businesses, ranging from digital start-ups to global life sciences companies. The two partners have invested £360m of capital, property and intellectual assets into the new venture, with a business plan supporting the creation of over 20,000 high value jobs in the regions over the next 10 years.

That portfolio includes Alderley Park in Cheshire, where I am the managing director and which is now home to a growing community of 70 SMEs, research charities and 150 start-ups or business operating a virtual model. The ongoing programme of investment at the Park has supported the expansion and development of our ecosystem of high specification labs, offices, scientific services and a programme of specialist business support. Alderley Park also has two venture funds based here, one of which, the £42m Greater Manchester and Cheshire Life Science Fund has already invested in 25 businesses across pharmaceuticals, biotech, diagnostics, CROs and medical devices.

The model here is about making sure we have genuinely world-class facilities for science and technology businesses at every stage of the lifecycle from start-up to large corporate within one location. The chances of any IP-based businesses succeeding are massively enhanced if it's operating in an environment where you have the right elements – by bringing these elements together in clusters in regional cities, we have seen how it can have a transformative effect. While we're extremely proud of the work we do, we are adamant that we need more sites like ours across the country – both to spur us on and to improve the number of scientific breakthroughs generated throughout the UK.

The business is aligned to the broader notion of rebalancing the UK economy so that it is not overly-reliant on London and the south east. That's not about the old north v south rivalry, to even east v west, but rather fostering competition for the greater good. Initiatives such as the Northern Powerhouse and Midlands Engine have science and technology as a priority area. At the same time, through devolution and other key initiatives, the government has made a more determined effort to unleash potential.

In the post-Brexit era, greater collaboration within the UK – a more joined up approach and greater awareness in Westminster of the economic potential in the regions – will be essential. The UK is a small country, with distances between the respective science and tech clusters insignificant compared to, say, the USA or China. The last thing we need to do is underplay what's on offer outside the traditional research hotspots.

Dr Chris Doherty

Dr Chris Doherty isleading the redevelopment of Alderley Park, Cheshire, UK, a major life science park. His role includes oversight of the development of the biotech cluster, forming and helping to grow biotech SMEs and CROs and developing science services that support the cluster. He has worked previously at major pharmaceutical companies, including AstraZeneca, Roche and Glaxo covering 25 years in multiple roles, with over 10 years at senior executive level.

Betting on the Bug: How the Microbiome Can Improve Reproducibility and Translatability

Investigators continually seek to improve the reproducibility of their results and the translatability of their preclinical work, while recognising that both goals pose challenges. In a 2016 *Nature* survey, over 70% of researchers said they were unable to reproduce another scientist's study and over 50% had failed to reproduce their own studies. For evidence that translatability from the lab to the clinic remains problematic, one needs to look no further than this fact: nine out of 10 drug candidates fail at the clinical trial stage.

How can investigators move beyond these obstacles and improve research outcomes? One of the very factors that can confound a study may also prove one of the solutions.

Harnessing the Microbiome

It is well known that the microbiome – the collection of microbes both in and on the body – can be a key contributor to study variability. By furthering our understanding of the microbiome's role in disease development, progression and treatment, and by improving our ability to control for this factor, the industry can take important steps toward improving study reproducibility and translatability.

The microbiome's influence on disease and therapy is an increasingly dominant subject, as evidenced by the sheer number of publications in which it's discussed. While it was first associated with diseases of the gut (understandably, since about 70% of human microbiota reside in the colon), studies continue to elucidate the microbiome's role in a wider range of conditions, including metabolic, cardiovascular, inflammatory and autoimmune diseases, as well as cancer. We're also learning that a shift away from a healthy microbiome composition can negatively impact the gut-brain axis, suggesting a link to neurological disorders. Additionally, we're now seeing that the microbiome can impact the efficacy of therapeutic agents. In one of many examples, a study published in *Science* found differences in the gut microbiome compositions of cancer patients who did or did not respond to checkpoint inhibitor therapy. It's also hypothesised that the microbiome may impact drug ADME and toxicity, though investigations into a connection are still in the early stages.

Given the significant potential for the microbiome to influence disease and treatment, it has become a key variable that investigators seek to control. However, when it comes to the most widely used research animal – the mouse – a variety of factors can impact the microbiome unintentionally. These factors, relevant to both a model provider's facility and a research institution site, include:

- Husbandry (type of housing/caging, cage density, feed type, feed sterilisation, bedding material, bedding change frequency, procedures for cleaning and sterilisation)
- Experimental and veterinary procedures (diet, compound administration, treatment with therapeutics)
- Mouse-specific factors (age, gender, genetics, stress, activity level, circadian rhythm, presence of disease)
- Mouse microbial profile (health standard, microbiome of the dam/mother, horizontal transmission of microbes, mode of birth)
- Animal care personnel (their microorganisms and pheromones)
- Environment (room temperature, humidity, air movement, light cycle)

With so many influencers, it is challenging to ensure consistency of mice microbiota over time, whether sourced from different providers or different breeding sites within a single provider. Globally harmonised health standards and strict operating procedures can help minimise differences; however, more often than not, health standard may not serve as the best proxy for the microbiome, since it only indicates with a high degree of certainty what is *not* present in the animal, rather than what *is* present.

A Holistic Approach

Though study reproducibility and translatability are complex issues requiring far more than a single solution, limiting the microbiome's impact is a critical step. Such efforts have tended to focus on the use of germ-free mice – those that harbour no micro- or macro-organisms. The blank slate of a germ-free animal provides a good foundational tool to add microbiota compositions for understanding host-microbiome interactions and for screening drug candidates.

Yet, while proper maintenance in gnotobiotic housing and strict, well-defined husbandry procedures should prevent wholesale changes to the microbiome (such as a loss or gain of organisms), factors such as those described earlier can lead to a shift in the relative abundance of microbial organisms over time. Additionally, since the microbiome is known to impact immune response, the blank substrate of a germ-free model does not fully reflect how disease progression or treatment will be influenced by the immune system, making translatability challenging. Thus, this type of reductionist approach is not always sufficient to control for the microbiome; instead, a more holistic approach is necessary.

A holistic approach to minimising the microbiome's influence may incorporate the use of germ-free mice but should also include a broader range of techniques. For instance, generating a mouse model with a defined microbiome allows the investigator to employ a host possessing the microbiota deemed relevant to his/her specific research application. In this approach, a germ-free model (knockout or humanised) can be colonised with the desired microbiota, then a cohort bred using strict housing and husbandry controls to maintain the desired microbial profile over time. As personalised medicine advances, the use of custom microbiota associations will also play a role in drug discovery. This gnotobiotic technique involves transplanting human faecal microbiota from a patient donor into a germ-free animal model, allowing the researcher to assess the effects of patient-specific gut bacteria on a wide range of biological systems and to test potential therapies.

Another viable approach is development of a mouse model with a wild-type microbiome, which has been shown to reflect a more natural immune response than a lab animal microbiome. The ability to study disease development and therapeutics in a host with a more naturally developed immune response can be advantageous, as demonstrated in a study by the National Institute of Diabetes and Digestive and Kidney Disease. Faecal microbiota from wild mice were transferred to germ-free lab mice, and a control group of germ-free mice gavaged with SPF (specific pathogen-free) material. When offspring from both of these groups were infected with influenza A, the survival rates varied dramatically: 92% for mice with a "wild-type" microbiome vs. 17% for the SPF mice.[1] Using a model of mutant/inflammation-induced colorectal tumorigenesis, the researchers also demonstrated that mice with the wild-type microbiota experienced less inflammation and a lower incidence of tumours as compared to the control group with SPF microbiota.

Improving research reproducibility and translatability will remain vital objectives for the drug discovery community. Enhancing our understanding of the microbiome's role in disease and advancing our ability to manipulate the microbiome through a holistic approach can serve as vital steps toward achieving these goals.

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1. Rosshart, et al. Wild Mouse Gut Microbiota Promotes Host Fitness and Improves Disease Resistance. Cell, volume 17, issue 5: 1015-1208 (2017).

Dr Alexander Maue

Dr Alexander Maue is Director of Microbiome Research Services at Taconic Biosciences. Previously he was Head of the Campylobacter Immunology Laboratory at the Naval Medical Research Center. Trained as an immunologist, his research focus was on enteric diseases and the development of vaccines and therapies to prevent illness.

Key Challenges and Potential Solutions for Optimising Downstream Bioprocessing Production

The term "optimise" is often applied to complex manufacturing, automation and business processes and implies that the most efficient function of all the elements of a process – technologies, sequences and procedures – has not been achieved. While no method can be perfect, every process can benefit from a careful consideration where new thinking, new techniques and new technology can yield significant improvements.

The dramatic growth in the use of biologics across multiple therapeutic applications and categories will only continue to increase. As the demand for these drugs accelerates, there are growing concerns about their cost and availability. Biologics manufacturers are investigating ways to address these concerns – and downstream production in bioprocessing operations is one such area.

Key Challenges in Downstream Production

Downstream production currently encompasses about 60 per cent of the total cost of producing a biologic drug. Finding ways to remove bottlenecks and improve yields in downstream could lead to more cost-effective production results, but there are several challenges associated with this goal:

Increased upstream yields: Significant investments have been made in the technologies and processes used in upstream processes with the goal of improving yields. Efforts to improve raw material characterisation, add single-use systems, perfusion systems and more precisely controlled bioreactors are leading to measurable increases in upstream yields. However, improvements in downstream throughput have not kept a similar pace to those for upstream, leading to potential bottlenecks in the end-to-end process.

A significant capital investment could be made to create larger chromatography systems to handle the increased production, but it would do little to accomplish the goal of cost-effectively aligning the productivity of downstream production with upstream yields. Loss from upstream to downstream: One of the fundamental structural challenges in biologic production is the approximately 30 per cent yields loss as harvest material goes through downstream purification. Any percentage of improvement in downstream recovery can contribute to improving the ultimate process yield for drug product of the target biologic.

Complexity of downstream production: Upstream productivity may be improving because it involves a more straightforward process. Once the target molecule and raw materials are loaded into the bioreactor, the process runs to completion with the appropriate testing and quality control.

Downstream production, on the other hand, involves multiple steps where the biological material is moved from harvest, centrifugation and polishing to multiple chromatography steps before reaching final fill and finish. Each step requires a unique set of resins and buffers among other materials; storage and production systems at multiple steps; and analytical and quality control sampling activities in parallel.

Finding efficiencies and economies of scale across downstream processing steps involves more complex analysis and optimisation. Improvements may be reached after investigating key aspects of current purification steps and technologies, including:

- Expanding the use of mixed-mode and multimode chromatography resins, including resins with targeted ligands with increased selectivity to more efficiently process targeted molecules
- Exploring ways to make chromatographic buffers more effective by using new kinds of additives, as well as utilising prepackaged single-use buffer materials to streamline buffer exchange steps
- Making wider use of data analysis tools to develop deeper insight into complex material interactions in downstream process steps, particularly as they relate to raw material characterisation

Improving Process Chromatography Technology

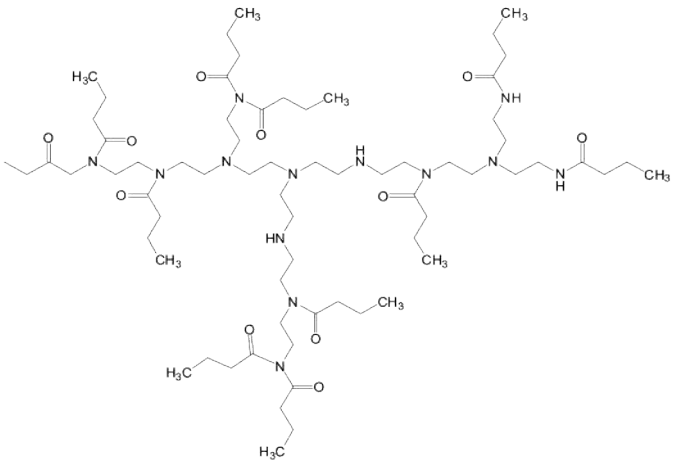
The ultimate goal of downstream optimisation is to improve recovery and therefore normalise, and potentially reduce, the cost per gram of protein produced. That means producing more drug product, using less time, with the same amount of resin and buffer material. One of the most effective ways to do this is by making better use of the newest generation of mixed-mode and multimode resins.

Biologic drugs are becoming more diverse, with more complex molecular structures. However, producing these precisely targeted drugs can also yield by-products that are very closely related chemically or biologically to the target molecule, with no therapeutic value.

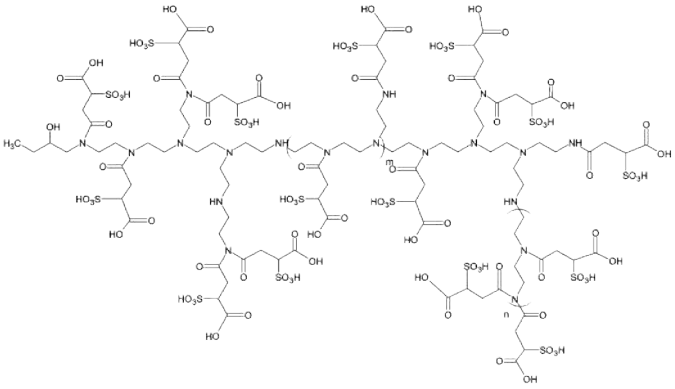
Separating undesired glycosylated molecules and aggregates presents major challenges, as they are likely to have limited differential binding to traditional ion exchangers and can coelute. Thus, the combination of increased upstream yields and more complex molecules calls for new approaches to chromatography resins. In response, chemistry suppliers have been focusing their efforts on process chromatography selectivity and efficiency.

The traditional solution to this type of challenge is to utilise multiple downstream ion exchanges. While it yields the targeted drug, the cost per gram of this approach can become prohibitive. More advanced methods of achieving effective selectivity are based on new ligand chemistries engineered to achieve very precise, selective interaction with the targeted protein. This approach increases selectivity while reducing the required number of steps, helping control process complexity and costs.

Targeted affinity chromatographic media are based on ligands tailored to interact with specific proteins, offering high selectivity for a target drug molecule. This can be a time-consuming approach if implemented for every new molecule; instead, mixed-mode and multimode approaches offer advantages to be considered.



Mixed-mode ligand structure: Mixed-mode media offer more interaction possibilities with the targeted drug molecule.



Multimode ligand structure: Multimode resins have the capacity to simultaneously interact with different sites or regions of the protein molecule.

Mixed-mode chromatography media are based on ligands that offer two or more interaction possibilities with the targeted drug molecule. The mixed-mode approach has proven to be effective and more productive in applications, such as intermediate and polishing steps, for purifying proteins based on differential salt-induced hydrophobicity. The advantage with the mixed-mode approach is that the same media can be used for different purification steps, modulated by solution conditions, such as using multiple buffers or multiple elution steps. However, newer mixed-mode resins have ligand chemistry that enable use of multiple, sequential interactions during the normal chromatographic process.

Multimode resins offer greater potential for efficiencies and improved yields. Rather than requiring multiple chromatographic purification steps, the simultaneous interaction makes it possible to separate very closely related proteins in a single step. This means it is possible to have the primary, secondary and tertiary interactions all happening at the same time. This simultaneous purification can occur without requiring additional intermediate steps, such as buffer exchange, buffer titration or dilution.

There have been studies showing that using a multimode or mixed-mode ligand with multiple interactions has the potential to boost chromatography yields while merging two process steps – and the ancillary time and costs associated with each step – ultimately impacting cost per gram.

For example, typical chromatography processes may first use a separate cation exchange step, then an ion exchange step. The yield is about 80 per cent pure after the first step, reaching upwards of 95 per cent after the second step.

With a multimode resin, it is possible to reach the 95 per cent purity in one step. Even with using a multimode resin in the column, it is more efficient to process 70 grams in one batch versus running 100 grams through separate cation and ion exchange steps. This is due to the overall higher throughput and the lower cost of materials, since this approach reduces the buffer consumption, types of filtration systems used, and ancillary costs associated with each chromatographic step. And since each step typically takes up to two hours, costly production time and labour can be cut in half.

Another method for optimising process chromatography is using the continuous chromatography method. In continuous multicolumn chromatography, the large column is effectively split into a number of much smaller columns that operate in series over a larger number of cycles. While product is loaded onto some columns, other columns in the set are going through the wash, elution and regeneration phases. Combined with resin optimisation and merging two chromatography steps into one, there is the potential for a three-fold improvement over traditional processes.

New Approaches to Buffers

Optimising the resin chemistry presents significant opportunities to improve downstream production. Improving the ways buffers are formulated and delivered to the end user can also positively impact productivity.

Traditionally, buffers have been very targeted: One type of buffer targets one type of pH in a column, then a different buffer is used to target a different pH. There is a move to more universal buffers that can be used in multiple process steps; while it's not possible to reuse the buffer, having to acquire, store and manage fewer buffers can help control costs.

There is also a greater focus on the use of additives to improve buffer performance. For example, in hydrophobic interaction chromatography (HIC), bioprocessors are working to fine-tune the selectivity of HIC functional groups. One method being explored is to use a select range of additives in the chromatographic media to improve the retention and selectivity of proteins as they move through the media, modulating their hydrophobic interaction and improving separation efficiency with decreased retention time, thereby improving throughput.

The advent of mixed-mode and multimode media and the increased selectivity they offer presents significant potential operational cost savings by eliminating intermediate purification steps that require additional time, materials, equipment and personnel.

Taking a more advanced approach to additives is one way buffers can be improved to optimise production. Another innovative advancement in buffer technology is the standardisation of buffer packages, predesigned for specific applications and delivered ready-to-use in single-use packaging.

Leading buffer suppliers are now implementing "buffer-on-demand" programmes designed to eliminate buffer-related costs in terms of labour, time and capital expenses from downstream production. Buffers are supplied in single-use packages – either pre-weighed and ready to use in solution, or as concentrates that can then be diluted and used in the columns.

This can eliminate capital expenses associated with buffer preparation tanks and equipment as well as storage space. It can also eliminate multiple buffer preparation, testing and validation production steps, directly impacting labour costs, time and management and documentation activities.

Chemistry suppliers who are providing these buffers on demand can work with the manufacturer to recommend and formulate very specific buffer materials, with stringent and well-documented materials characterisation, so that the biologics manufacturer can be assured that the buffer's performance in the chromatographic step is always on-target.

Enhancing Use of Data Analytics Tools

One area where the biopharmaceutical industry lags behind other manufacturers is the aggressive use of data and predictive analytics to mine for and uncover ways to improve productivity, process yield and costs. The industry is making significant investments to improve its use of data.

Currently, much of the focus is on using process data to optimise a process, then reliably repeat that batch by reaching the perfect balance of process parameters. These efforts are being conducted for both upstream and downstream production; however, much of the focus is on the process data itself, somewhat in isolation.

There is an opportunity to expand the application of data analytics beyond the process to more precisely and completely characterise the raw materials used in both upstream and downstream production, then integrate the data into overall optimisation efforts. In many cases, the biologics manufacturing sites are simply using standard certificate of analysis (CofA) data received with the delivery of chromatography resins, buffers and other production materials.

Raw material variability presents serious issues impacting downstream efficiency, resulting in long investigations and potential delays in making drugs available for patients. Leading chemistry suppliers are implementing more advanced characterisation efforts that provide manufacturers with further insights into the variability of materials within the integrated supply chain. These include supplying e-datasets, such as:

- CofA data for all raw material lots manufactured
- Manufacturing in-process data
- In-test actuals for conforming specs
- Stability testing interval data

This approach could enable a biologics manufacturer to more accurately assess and predict the process performance of any given raw material ahead of its use. This is a more holistic and data-driven way to assess the total impact of other downstream optimisation steps, such as adopting more targeted resins or implementing the use of novel additives in buffers.

"Buffer-on-demand" programmes can eliminate buffer-related costs in terms of labour, time and capital expenses from downstream production. Buffers are supplied in single-use packages – either pre-weighed and ready to use in solution, or as concentrates that can be diluted and used in columns.

New Potential for Downstream Optimisation

The ultimate goal for downstream optimisation is clear: controlling or even reducing the cost per gram of value biologic drugs. Many of the more expensive elements of current downstream process steps, such as chromatography media, can be utilised more efficiently by investigating how newer, state-of-the-art materials offer ways to condense and streamline process steps.

Each downstream process has requirements unique to each target drug. Working with suppliers of downstream materials who have deep insights into how process chemistries and raw materials perform can be a highly productive way to advance downstream biologic optimisation.

Nandu Deorkar

Nandu Deorkar, PhD, MBA, is Vice President, Research & Development for Avantor. During his more than 25-year career in materials technology research & development, Dr Deorkar has worked on various aspects of chemical/polymer R&D, drug development, formulation, drug delivery technologies, process development, and technology transfer. Dr Deorkar earned his PhD in chemistry from the Indian Institute of Technology, Bombay, and his MBA from Fairleigh Dickinson University, New Jersey (USA).

Claudia Berron

Claudia Berron is the Vice President of Strategy and Market Development for BioPharma Production at Avantor. Berron had two decades of experience in B2B strategic marketing covering ideation, value proposition strategies, market segmentation, marketing and sales plan, through product launch. Berron holds an MBA degree from the University of North Carolina, Kenan-Flagler Business School, Chapel Hill, and a BA degree from Monterrey Institute of Technology in Mexico City.

New ambr® 250 Modular Bioreactor Vessel Launched for Cell and Gene Therapy Applications

- **New vessel is designed for gentle stirring and optimum growth of cell lines**
- **Cost-effective, scalable process development of cell and gene therapies**

Sartorius Stedim Biotech has launched a new vessel for its ambr® 250 modular benchtop automated mini bioreactor system. The single-use vessel has been specially designed for therapeutic cell lines and offers the potential for accelerated process development of cell and gene therapy applications and scale-up into cGMP single-use bioreactors and bags.

The new unbaffled vessel design with a large pitched blade impeller has a working volume of 100–250 mL and provides an environment for gentle agitation and mixing without sedimentation, allowing optimal growth of single cell suspensions, cell aggregates or adherent cells on microcarriers. In trials with leading regenerative medicine companies, the new mini bioreactor has shown better cell culture performance compared with less predictive spinner or T-flask models, enabling rapid process optimization and improved scalability to larger bioreactors.

To further support culture of these cell lines, ambr® 250 modular systems also feature a new state-of-the-art motor (100rpm–4,500prm), ideal for the lower stirrer speeds required by delicate therapeutic cell lines. The system is suitable for culturing cell lines including such as HEK293, CAR-T and other therapeutic cell lines, including a range of stem cells, enabling scalable media and supplement optimization, as well as process development of cell and gene therapies.

The bioreactor system is available with optional BioPAT® MODDE software for DoE (Design of Experiments), to support QBD (Quality by Design) for scale-up to SSB's BIOSTAT® STR stirred bioreactors and scale-out to BIOSTAT® RM TX rocking bags suitable for cGMP production of autologous and allogeneic cell and gene therapies.

Figure 1: The new mini bioreactor unbaffled vessel design with a large pitched blade impeller, enables a gentle stirring and optimum growth of cell lines.

"The ambr® 250 systems are established as the biopharma industry standard small scale model for biologics process optimization, and we have been approached by numerous scientists developing cell and gene therapies to extend our technology for these applications," explained Dr. Barney Zoro, ambr® Product Manager at Sartorius Stedim Biotech. "We have responded by designing a new vessel for the ambr® 250 modular system to provide a single-use platform, with a clear scalable pathway to our bioreactors and bags for clinical production of regenerative medicines. Researchers utilizing this workflow, can benefit by reducing time-lines and minimizing manufacturing costs, thus allowing larger patient numbers to access these revolutionary therapies sooner," Zoro added.

Dr Barney Zoro,
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Figure 2: The ambr® 250 modular bioreactor system can now even be used for cell and gene therapy applications.

Gene Therapy: Bubble Boy and Beyond

Gene therapy, i.e. the delivery of DNA or RNA into cells to treat disease, is at the cutting edge of medical research, and often makes the headlines. More importantly, recent developments have demonstrated the potential clinical impact that gene therapy can have in treating rare inherited diseases, as well as potentially some of the world's most prevalent conditions.

In April 2019, gene therapy made global news when the results of a Phase I/II safety and efficacy trial using gene therapy to treat infants diagnosed with X-linked severe combined immunodeficiency (SCID-X1) were published in *The New England Journal of Medicine*. The study provides the latest evidence of the potential for using gene therapy in modern medicine.

SCID-X1 is an inherited disorder of the immune system which results in major abnormalities in white blood cell production and function, including devastatingly low levels of T cells and natural killer (NK) cells, together with non-functional B cells. The condition was brought to the attention of the world in the 1970s with photos of David Vetter, who, in the absence of a suitable bone marrow donor (the only effective treatment at the time), had to be kept in a sterile isolation chamber and later became known as the Bubble Boy.

SCID-X1 is caused by mutations in the *IL2RG* gene, which encodes the common gamma chain, an essential component of a number of cytokine receptors which control the development and function of T cells, B cells and NK cells. In the present study, self-inactivating, HIV-1 derived viral vectors, containing *IL2RG* cDNA under the control of an EF1α promoter, were used to transduce blood stem cells (CD34+ cells derived from patient harvested bone marrow). The transduced cells were then infused into each of the patients following treatment to facilitate reconstitution.

The resulting effects on the patients' immune systems were striking. Seven of the eight patients on the trial displayed normal levels of the various T cell populations within two to four months following infusion. NK cell populations were also normalised in many of these patients. Furthermore, protective antibody responses against various infectious diseases were shown in a sub-cohort of patients who received vaccination following gene therapy, indicating the presence of functional B cells.

This is a significant success, especially compared to the earlier attempts at treating SCID-X1 patients with gene therapy, which were reported to be associated with the development of leukaemia in some patients, or which failed to restore certain populations of immune cells in others (meaning a lifetime of immunoglobulin injections).

Although the authors of this study are careful to remind us that long-term follow-up will be needed in order to assess the durability and long-term safety of this treatment regime, the study gives an indication of how far things have progressed in the gene therapy sector.

Indeed, whilst gene therapy is generally considered to have been a very recent development, fundamental research in this sector goes back over half a century, with early proof of concept experiments demonstrating the replacement of defective DNA in cells using viruses. Some of the earliest gene therapy trials date back to the 1990s, and interestingly, these too were aimed at the treatment of a form of SCID (albeit ADA-deficient SCID which results from a mutation in the gene encoding adenosine deaminase). ADA-deficient SCID and SCID-X1 are, of course, exemplary targets for gene therapy, given they result from mutations in a single gene.

The present study comes at a time of significant activity in the gene therapy world. Research into gene therapies continues apace. In early May 2019, a new gene editing company, Verve Therapeutics, co-founded by Harvard academic Sekar Kathiresan, was launched. The aim was to develop a treatment to significantly reduce the risk of heart attacks, the world's leading cause of death, with a single injection. The therapy uses nanolipids to target the enzyme PCSK9 (proprotein convertase subtilisin kexin 9), which is involved in the production of so-called 'bad cholesterol', (also known as low-density lipoprotein).

The therapy was designed to shut down one of the two copies of the PCSK9 gene in a patient, mimicking a mutation which occurs in a subset of the population who have naturally low cholesterol and a reduced risk of heart attacks. The initial trial will be conducted in patients with homozygous familial hypercholesterolaemia (HoFH), who have statin-resistant high cholesterol levels. If successful, the treatment could be used more widely to significantly reduce the occurrence of heart attacks and associated fatalities.

Such advances are not restricted to the laboratory, with increasing numbers of potential gene therapies entering clinical trials and then into the clinic. In a recent statement, the FDA announced that it expects to approve between 10 and 20 new cell and gene therapy products per year from 2025 onwards, resulting from an anticipated 200 investigational new drug (IND) applications per year from 2020 onwards.

This increase in activity is said to reflect "a turning point in the development of these technologies and their application to human health", which has been driven, in part, through the adoption of adeno-associated viral (AAV) vectors for the delivery of gene therapy agents. The FDA has likened this increase in activity to that seen in the late 1990s with antibody therapies, following the development of platforms for producing fully human monoclonal antibodies.

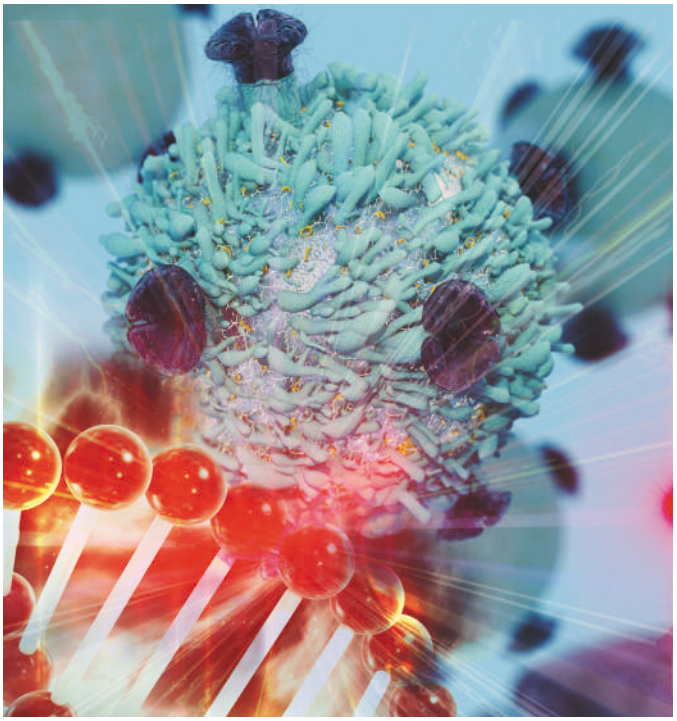
At the end of 2017, the Alliance for Regenerative Medicine reported that there were 946 clinical trials underway investigating gene and cell therapy products, with new trials constantly being announced. In February 2019, for example, Gyroscope Therapeutics successfully administered the first dose in a clinical trial which investigated the safety and efficacy of their gene therapy candidate for the treatment of dry age-related macular degeneration (AMD), one of the leading causes of blindness in the world. Although traditionally gene therapy has been seen solely as a potential modality for the treatment of monogenic disorders, should this investigation be successful, it could widen our view of the applicability of gene therapies (given that dry-AMD is a multifactorial disease).

As shown by the data generated from the SCID-X1 patient trials discussed above, these advanced therapeutic modalities are showing success in the clinic. Novartis' Luxturna, an AAV vector delivering a functional copy of the RPE65 gene to cells of the retina, has now been approved for the treatment of inherited retinal dystrophy in both the US and Europe, and follows in the footsteps of Strimvelis (an approved gene therapy for the treatment of ADA deficient-SCID).

With this flurry of successes has come (renewed) interest in gene therapy, highlighted by recent multi-billion dollar acquisitions by big pharma companies. For example, AveXis Inc. (who developed Zolgensma, a spinal muscular atrophy treatment candidate) was acquired by Novartis in 2018 for $8.7 billion. More recently, Roche paid $4.8 billion for Spark Therapeutics and its haemophilia treatment candidates.

This is good news for SMEs too, of which there are many who are building on the foundational research of the last few years to develop new and exciting gene and cell therapy products. The United Kingdom in particular is evolving into one of the leading places for the development of gene- and cell-based therapies. A world-leading combination of academics, large pharma, manufacturing facilities, contract research organisations, innovation agencies and pool of active investors, together with dedicated and co-ordinated advanced therapeutics research centres has provided the perfect ecosystem for these emerging companies to flourish.

Whilst the hive of activity should encourage researchers and innovators working in the gene therapy sector, the challenges



facing this sector should be kept in mind. Even positive clinical trial results and regulatory approval will not ensure success, as exemplified by the withdrawal of Glybera, which became the first gene therapy product to be approved in Europe in 2012. Eye-watering price tags (Glybera reportedly cost €1 million per treatment), small patient populations, and complex regulatory exercises are just a few of the challenges facing the industry.

There appears to be consensus in the industry that innovation in commercial-scale manufacturing methods will be key in order to bring costs down and facilitate availability of these often life-changing therapeutics to patients. The production of AAVs has, to date, been one of the key hurdles to overcome. Unlike other viruses, AAV requires an additional 'helper' virus in order to allow them to replicate in cells. Whilst methods have been developed to eliminate the need for these helper viruses, through the generation of producer cell lines expressing key helper virus genes, empty vectors (i.e. AAV particles not containing the gene therapy construct) is still a major issue requiring expensive and time-consuming purification methods in order to produce the required titres. Importantly, SMEs developing and trialling gene therapy products should always look to the future to ensure that their methods/processes are scalable when necessary.

As well as the complex manufacturing methods and state-of-the-art facilities required to produce gene therapies (in particular, the viral vectors used to deliver nucleic acid), the costs of these agents also reflect the lengthy and expensive journey of a gene therapy from the bench to bedside. Companies in this sector should carefully consider and protect their innovations to ensure their efforts are not only safeguarded, but also recompensed.

Whilst patent protection of the gene therapy products themselves (i.e. vectors, nucleic acids, formulations, etc.) is apparent, companies should also bear in mind innovations in their manufacturing processes and the like. As indicated above, manufacturing innovations are likely to be key to the success of gene therapy; protection of these aspects can be a useful strategy to maintain exclusivity (even beyond the patent life of the product) and generate additional income through licensing. Of course, in order to obtain a patent, one must adequately disclose the innovation in the patent application, and as such, timing is likely to be crucial.

The gene therapy sector faces numerous challenges, not least from a regulatory, safety and, indeed, ethical perspective. Despite this, as the SCID-X1 study has shown, gene therapy represents one of the most powerful treatment modalities available to date, which, in many cases, can provide life-changing results. A concerted effort from those in academia, industry and policy will be required to continue this success, making safe and effective gene therapies available to wider patient populations. The current excitement around this technology is likely to attract a flurry of new players to the market and, as such, intellectual property will be a vital foothold for those wishing to establish their position in this field.

Indeed, the recent UK ATMP Investor Day (co-sponsored by Mathys & Squire) gave 11 such companies the opportunity to pitch to life sciences investors. Partner, Anna Gregson, who spoke at the event and has extensive expertise in this technical field, noted: *"The UK has an incredible ecosystem for cell and gene therapy research – with supporting organisations such as the Cell and Gene Therapy Catapult, an active investment community and a thriving research community. All these factors together enable the UK to produce world-class cell and gene therapies; as evidenced by the calibre of the SMEs who pitched at the UK ATMP Investor Day. It is a really exciting time to be involved in cell and gene therapy research, and I am thrilled to play a part in supporting SMEs in this space!"*

Anna Gregson

Anna is a partner in Mathys & Squire's biotech team. She has over 10 years' experience working with a diverse client base; from university technology transfer organisations to international corporations. Anna's expertise covers a wide range of biotechnology and life sciences subject matter, with a particular emphasis on the cell and gene therapy space, including CAR T cells, iPSCs, neural regenerative medicine, cell culture technologies, and viral/non-viral gene therapy vectors.

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Dean Houston

Dean is a trainee patent attorney in Mathys & Squire's biotech team. He has a strong background in the cell and gene therapy sector, having completed his PhD at The Roslin Institute in Edinburgh. Dean has experience in the global prosecution of patent families across a diverse range of technologies, including therapeutic toxins, antisense oligonucleotides, vaccines and methods of neuronal stem cell culture.

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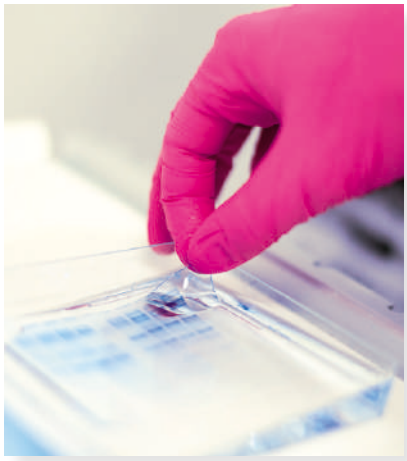

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Challenging Proteins Made Easy

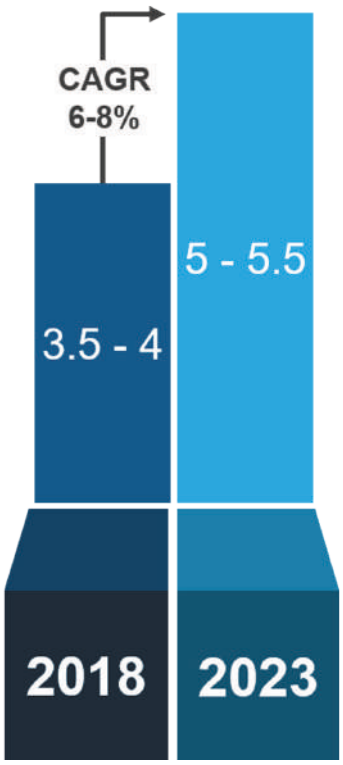
Changing State of Clinical Trials in the Digital Era

The life sciences industry is undergoing a fundamental change in its business model. The shifts from blockbuster drug to precision medicine and from volume-based to performance-based models has disrupted the life sciences ecosystem. Additionally, the cost of clinical trials is continuing to climb; the cost of bringing a drug to market currently stands at ~$2 billion (60–65% is attributed to clinical development), which has nearly doubled in the last decade.

Clinical trials are conducted in multiple phases, with Phase III trials requiring a larger pool of patients and being significantly more expensiveand complex than Phase I trials. Clinical trials fail for a variety of reasons, including failure to recruit enough participants, mid-trial patient drop-out, and poor data collection methods. Trials that fail at a later stage prove more costly for both the company conducting the trial and the patients. Other than the cost concern, patient safety and study quality are absolute imperatives for all clinical trials.

In order to move away from this high-cost R&D model, as well as to enable closer engagement with patients, pharma and research companies are turning to digital technologies to transform clinical development.

Global clinical trial technologies market (US$ billion, 2018–2023)

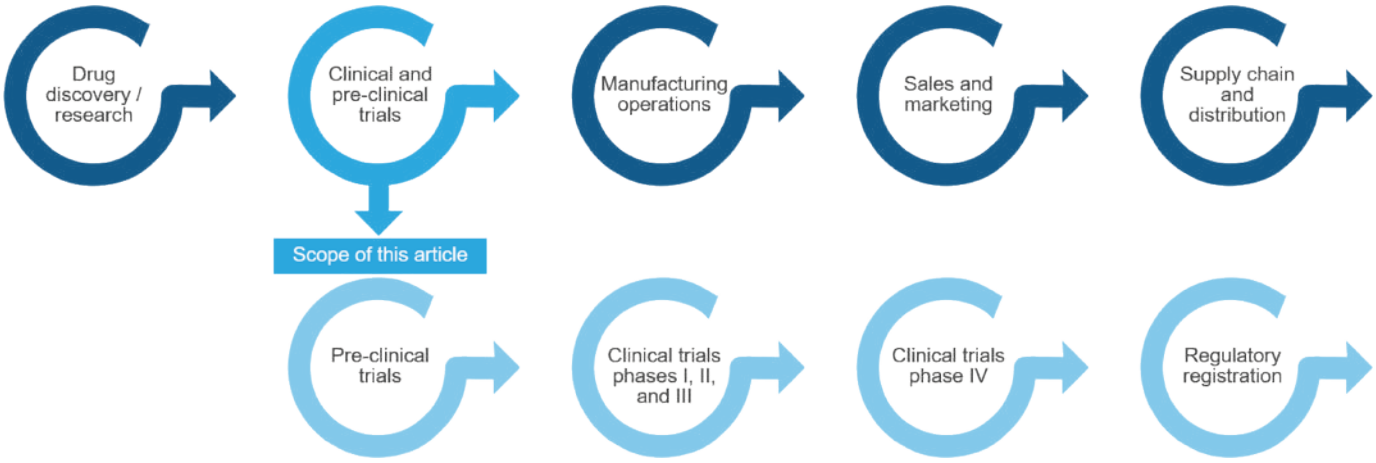


Market Overview

The global clinical trial technologies market (comprising products and solutions, services, and hardware) is expected to grow at a compound annual growth rate (CAGR) of 6–8% over 2018–2023, given the rise in the number of clinical studies and increase in regulatory and safety standards. Countries worldwide are amending clinical trial guidelines to keep pace with the scale and complexity of clinical trials and to ensure appropriate use of technology. Adherence requires improvements to clinical trial design, conduct, oversight, recording, and reporting.

Technology adoption in clinical trials helps to:

- Accelerate the time-to-value, while maintaining quality and safety
- Minimise data fragmentation
- Simplify integration and reporting across multiple clinical trial applications
- Move away from a point solution-based approach (targeted at single research instances) to a unified environment
- Identify risks and trends faster, leading to proactive interventions in functions where potential problems are identified



Focus on Products
The products and solutions segment accounts for more than 60% of the clinical trial technologies market, followed by hardware (15–25%) and services (15–20%).

Technology products and solutions play a key role in accelerating time-to-market and improving trial outcomes. Most of these systems are plug-and-play, with in-built automation capabilities, and they help enterprises optimise costs. They also minimise the need for data reconciliation and standardisation, as they offer a standardised metadata format. Core components of the products and solutions segment include clinical operations / trial management, data capture and management, and site solutions.

Key segments of clinical trial products and solutions market

The clinical trial management system (CTMS) is the most popular solution, and the industry is likely to increase its CTMS investments at a 10–15% CAGR over 2018–2023, driven by increasing demand for CTMS to manage complex protocol designs, as well as the growing demand for clinical trial documentation.

North America is the biggest market, accounting for nearly two-thirds of the overall global clinical trial technologies market. Robust infrastructure, widespread use of mobile devices, and an enabling regulatory framework are creating opportunities for adopting other digital technologies in clinical trials in North America. APAC is the fastest-growing market, expected to record a CAGR of 12–15% during 2018–2023 on the back of increasing clinical trial activities,improving internet connectivity, and growing mobile device acceptance. South Korea, China, and Japan are key markets in this region.

Market Trends / Challenges
Unification of the clinical trial estate (platform-led):
Data residing in silos is not very useful for the drug discovery process, as it does not give a holistic understanding of trial outcomes. An integrated view of clinical trial records is essential to understand the actual effects of clinical trials as well as to apply data analytics capabilities, to be able to predict possible outcomes. Additionally, standardisation and integration are helpful during regulatory registration and submission. Product vendors are responding to the market trend by moving away from a point solution-based approach to a unified environment. For instance:

- Oracle's cloud-based eClinical platform, Clinical One, combines capabilities such as randomisation and supply management, data management, CTM, portfolio planning, budgeting, resourcing tracking, risk-based monitoring, data review, query reconciliation and management, safety management, and safety signal detection.
- Veeva Vault Clinical Suite's cloud-based software platform combines a clinical data management system [including electronic data capture (EDC) and coding], CTMS, electronic Trial Master File (eTMF), and study start-up to deliver a suite of clinical cloud applications.
- Medidata's cloud-based clinical trial platform, Rave, combines capabilities such as data capture, data management, trial planning, trial management, and analytics.

The hallmarks of these and other similar platforms are a combination of products and services in a utility-based construct, where customers can plug in and plug out based on their needs.

Clinical embraces the cloud:
While life sciences firms have been putting an increasing number of enterprise applications and data on the cloud, they have been hesitant to do so with R&D and clinical data. This is now slowly changing, as life sciences firms have been adopting the cloud in core processes and for clinical and R&D workloads. For instance, Accenture, Merck, and AWS partnered in 2018 to announce a cloud-based research platform. Key drivers for cloud adoption include storing data virtually (managing storage growth), managing and analysing data real-time, enabling a unified data view to make informed strategic decisions, and designing and deploying disaster recovery solutions.

Moving from real-world data to real-world evidence:
Evidence-based clinical trials are necessary to enable a shift from blockbuster drug models to precision medicines. This change requires integrating individual clinical expertise with external clinical evidence from systematic research. To facilitate this change, life sciences enterprises are starting with low-hanging fruit such as EHR (electronic health records)-to-EDC integration through various sites to unlock value from data. In February 2019, Pfizer teamed up with Ochsner Health System to develop digital tools for EHR-to-EDC integration.

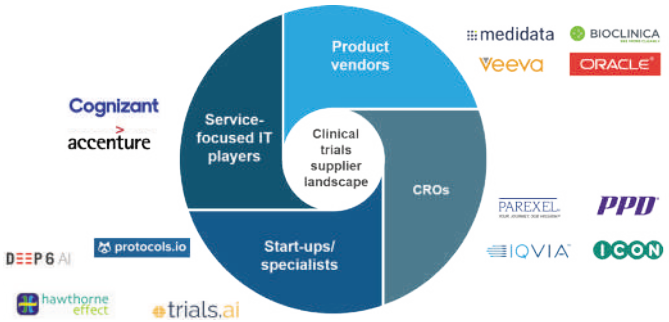
However, to truly move the needle from data to evidence, enterprises need to review trial design, protocols, and treatment methodologies from the perspective of all industry stakeholders (patients, payers, providers, regulators, etc.). This, combined with historical evidence, would accelerate precision medicine development.

Patient-centricity comes to the forefront:
Withover four-fifths of clinical trialsfailing to recruit enough patients and nearly a third of patients dropping out of studies after they enroll, patient recruitment and retention have become key priorities in clinical trials. Trials have started deploying technologies such as telehealth and remote monitoring to eliminate the need for patients to travel to investigator sites. Additionally, to minimise dropouts, patients are kept invested and engaged. Technology and data analysis can be used to driveretention and adherence solutions by identifying at-risk patients. With the help of targeted insights, trials can take a more proactive approach to manage patients' needs and expectations. For instance, Novartis and GSK have scaled the use of Apple ResearchKit (ARK) in clinical trials, as part of their efforts to bring about digital transformation across their businesses. ARK allows clinical trial participants to enter their own data remotely, including via sensors that collect movement and symptom data, removing the need for patients to visit the clinic.

Partner Ecosystem

The supplier landscape is characterised by diverse players, including pure-play product vendors, service providers, contract research organisations (CROs), and specialists – each with a unique value proposition, differentiated portfolio coverage, and different levels of market impact.

Clinical trial supplier landscape



- Product vendors' focus on technology (such as analytics and automation) and next-generation offerings, coupled with their robust platforms, serves as a key differentiator.
- Service-focused IT integrators have strong business plus IT play, with substantial capabilities to deliver stand-alone platforms or add wrapper solutions in the clinical trial technologies market.
- CROs demonstrate on-the-ground clinical trial execution capabilities, with high investment in real-world data assets.
- Start-ups/specialist firms offer point solutions and help achieve specific clinical trial objectives, such as patient recruitment and therapy adherence.

State of Technology Adoption in Clinical Trials

The pharma industry is under immense pressure to develop innovative medicines; demonstrate differentiated value to patients, providers, and payers; and reduce costs and time-to-market to maximise return on investment. To achieve this, companies have started embracing digital technologies across the clinical trial process. Reducing clinical trial costs and enhancing efficiency through early identification of trial challenges (patient recruitment, data monitoring, and data reporting) are the key priorities for trial sponsors.

Though in a nascent stage of adoption, these technologies have the potential to drastically change the way in which clinical trials are conducted.

Areas with high adoption potential across the clinical trial process

Clinical trial processes	Trial design	Trial startup	Trial conduct	Study closeout
Processes	• Collection of patient/investigator input • Collection of medical data to inform trial design • Genomic data sourcing • Protocol designing	• Site selection • Patient recruitment and informed consent • Regulatory submissions	• Clinical trial monitoring • Recording patient-generated data • Data capturing and reporting • Protocol and therapy adherence	• Cleaning and automating data • Analyzing study data to investigate data correlations, trends, and results

- **Trial design:** This involves application of digital technology for creating a clinical trial protocol (objectives, patient profiles based on medical characteristics, milestones, and patient outcomes). Key technology bets include EHRs, big data and predictive analytics, AI, and mHealth (mobile health technologies).

  In January 2018, Sanofi collaborated with TriNetX to use data from EHRs to reduce the complexity of trial design, increase recruitment success, help streamline the work of trial investigators, and reduce time from protocol to results. In the same year, Pfizer followed Sanofi's lead and partnered with TriNetX to harness real-world data for clinical trial optimisation.

- **Trial start-up:** This involves digitalisation of study start-up to enable investigators to predict the best-performing sites,find patients who want to participate in clinical trials, and identify patients with genomics and real-world evidence. Key technology bets include web and social media, big data and predictive analytics, machine learning (ML) and natural language processing (NLP)-based applications to improve matching results, and cloud-based technologies.

  In December 2018, Novartis entered into a partnership with TrialSpark to develop a patient-centred model for clinical trials that enables patients to enroll in studies from their doctors' offices, rather than selected research centres. In 2017, Oracle acquired goBalto to accelerate clinical trials by streamlining and automating the selection of the best-performing clinical research sites, leveraging cloud solutions.

- **Trial conduct:** This involves technology solutions to allow physicians to keep up with patient/trial data efficiently, monitor medication adherence, and track real-time progress and detect anomalies in trials. Key technology bets include EHRs, internet of things (IoT) platforms, mHealth (smartphones and tablets), wearables, telemedicine, predictive and advanced analytics, and cloud platforms.

  Leading pharma companies such as Roche and GSK are using mobile-based platforms for real-time patient monitoring, data reporting, and process capturing. The use of mobile devices reduces the need for frequent patient visits and brings down the associated costs.

- **Study closeout:** This involves cleaning and automating raw data to reduce the time spent after trial conduct in preparing results for submission, leading to shorter and cost-efficient trials. It also involves enabling automatic analysis of study results to extract meaningful correlations/trends. Key technology bets include artificial intelligence (AI), advanced analytics, and cloud platforms.

Future of Clinical Trials

Digital technologies have the potential to transform clinical trials. At present, pharma companies are investing in piecemeal solutions (EHRs, wearables, etc.) to bring about incremental improvements to existing clinical trial processes.

The three key challenges for technology to solve are **patient-centricity, process,** and **innovation**.

There are only a few large pharma companies that have set up innovation groups / allocated budgets to pilot transformative approaches (virtual trial, adaptive trial design, etc.) that could change the way trials are conducted. In March 2018, Novartis collaborated with Science 37 (a mobile technology and clinical trial start-up) to initiate new clinical trials that would blend virtual and

Present state	Future state
Patient-centricity	
• Patient recruitment is done in isolation from the core trial needs, resulting in inaccurate trial outcomes • Patients drop off in between the trial process • Non-adherence to medication, leading to incorrect trial results	• Automated recruitment to match patient conditions to trial needs. Digital technologies can support the recruitment of a more diverse and representative study population • Absolute adherence to medication/therapy via consistent monitoring through medical devices • Minimal patient drop-off on account of active patient engagement [including the incorporation of patient inputs in the trial process and a BYOD (Bring Your Own Device) approach to electronic patient-reported outcome (ePRO)]
Process	
• Delay in drug supply or inaccurate drug supply • Version control errors and duplications • Inexact trial protocol design	• Automated drug delivery using IoT • A digital platform that integrates and validates clinical trial data through EHRs, insurance claims, wearables, and other sources of electronic data in real time, to provide a unified view of data • Protocol feasibility and patient recruitment by mining EHRs and patient records • Use of adaptive trials, which involves modifying study protocols in predetermined ways (based on interim patient data) to eliminate unanticipated risks that undermine successful drugs or extend development timelines
Innovation	
• Paperless tracking of clinical trial supplies • Capturing of patient-centred endpoints • Remote monitoring of patient adherence • Workflow automation of a few repetitive/routine activities	• Pragmatic trials in uncontrolled, real-world clinical settings with typical patients and by qualified clinicians to improve the effectiveness of an intervention • Master protocols that incorporate specific design features to ensure patient safety and obtain quality data that may support drug approval; master protocols are specifically relevant for umbrella and basket trials • Virtual trials to enable patients to participate in studies remotely, enhancing patient access and engagement and speeding up cycle times for products in development • Use of real-world data that has been collected from sources such as EHRs, claims data, and patient-generated data, instead of recruiting patients to collect data for a trial

traditional models, with an increasing degree of decentralisation toward a "site-less" model. In the same year, ICON releaseda technology platform (ADDPLAN® neo) for design, simulation, and analysis of adaptive clinical trials.

The Path Forward

Digital technologies have the potential to disrupt every stage of the clinical trials process – from matching eligible patients to studies to monitoring adherence and data collection. However, the overall life sciences industry has been slow to digitise clinical trial processes. Even the most advanced enterprises are currently piloting technologies in different areas of clinical development, focusing on point solutions such as data capturing and data management, rather than the transformative approaches (virtual trial, adaptive trial design, etc.) that could change the way trials are conducted.

While most organizations are still in the early stages of exploring utility of digital technologies in clinical development, the industry needs to act immediately to devise a robust strategy to harness the full potential of digital in clinical development (such as better engagement with patients, deeper insights, and faster cycle times for products in development). To achieve this, pharma and research companies will need to overcome several internal and external challenges. Updated data infrastructure and governance, engaging early with regulators to discuss new technologies, and developing a measured approach to evaluating and implementing technologies within their organisations, can solve for some of the challenges.

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A Statistical Approach to Improve Compound Screening in Cell Culture Media

The Chinese hamster ovary (CHO) cell line is widely used for the production of recombinant proteins due to its high growing capacity and productivity, as well as other cell lines derived later than CHO. Adapting cell culture media for each specific cell line is a key to exploiting these features for cost-effective and fast product generation. Media supplementation is generally addressed by means of one-factor-at-a-time or classical design of experiments approaches, but these techniques may not be efficient enough in preliminary screening phases. In this study, a novel strategy consisting of folding over the Plackett–Burman design was used to increase cell growth and trastuzumab production of different CHO cell lines through supplementation with non-animal recombinant compounds. Synergies between compounds could be detected with a reduced number of experiments by using this methodology in comparison to more conventional fractional factorial designs. In the particular case reported here, the sequential use of this modified Plackett–Burman in combination with a Box-Behnken design led to a 1.5-fold increase in cell growth (10 x 10^6 cells/mL) and a two-fold in trastuzumab titre (122 mg/L) in suspension batch culture.

Introduction

Today, the most extended practice in the culture of mammalian cells is the use of commercially available chemically defined (CD) and animal-derived component-free culture media. These media have been optimised to support cell growth and recombinant protein production and are usually very complex. However, their composition is not known since they are proprietary. When used for a specific cell line, they may not support all the specific requirements for it, and therefore, additional supplementation of these media with other compounds at optimal concentrations may provide substantial improvement in cell performance.

The CHO cell line is the preferred mammalian cell host for the production of recombinant proteins[1]. Among the multiple benefits of using this system, it should be highlighted that CHO cells can grow in chemically defined and serum-free media enabling standardisation of the production process and Good Manufacturing Practices compliance[2]. Also important, CHO cells are easily adapted to grow in suspension culture, which is preferred for large-scale culture[3]. The glycosylation pattern of recombinant proteins produced in engineered CHO cells is very similar to that of human cells. Consequently, protein products can be used in humans since they are non-immunogenic and bioactive[4]. CHO cells change their chromosome composition at random and frequently, which contribute to their easy adaptation to different culture conditions and the possibility of finding high producer clones through screening[5]. Nowadays, recombinant protein titres from CHO cell culture have improved significantly, reaching the 5–10 gram per litre range[6,7]. In this regard, media supplementation plays an important role in searching for best production conditions since product titre is proportionally related to the number of viable cells in almost all cases[6,8]. In this context, different non-animal-derived recombinant additives were selected according to their capacities to improve cell culture conditions: r-insulin, r-transferrin, tween 80, selenium, r-albumin, fatty acids, synthetic cholesterol, and (+)-α-tocopherol[9]. Among the most important features of these compounds, insulin acts as a mitogen and growth and maintenance factor in many cell types[10]. Transferrin is a glycoprotein extensively used as an iron chelator in serum-free media and its depletion causes severe inhibition of the cell growth[11]. Tween 80 is a non-ionic surfactant and emulsifier frequently used to prevent cell clumping[12]. Interestingly, it has also been recently reported that it improves protein production in different mammalian cell types[13]. Selenium is an essential trace element for normal cell growth and development. It is incorporated into enzymes that protect cells by reducing peroxides fundamentally acting as an antioxidant[14]. Human serum albumin can also act as an antioxidant, as well as playing an important role in binding and transporting physiologically important ligands[15]. Fatty acids and synthetic cholesterol are normally required for cell growth since they are the main constituents of the phospholipidic cell membrane[16]. Finally, (+)-α-Tocopherol acetate, also known as vitamin E, is an important lipid that helps to stabilise the cellular membrane and span the viability of the cell culture[17].

PRACTICAL APPLICATION

In animal cell-based processes in the biopharmaceutical industry, media supplementation tailored to each cell line is now a days a very relevant component for production optimization. A broad approach followed toward media optimization is to first use a screening methodology, normally a Plackett-Burman design (PBD), to discard compounds with no or negligible effects on cell culture. However, the classical PBD cannot distinguish between the effects due to individual factors and interactions, which may be critical in next experimentation phases. In this work, the authors propose a folding-over strategy of the PBD in order to be able to detect these synergies between factors. Also, a comparison between the classical PBD and the technique here proposed is shown to have significant differences. By using this methodology, the authors envisage a window of applicability for media supplementation since more robust decisions can be undertaken during the phase of compound screening.

The assessment of such a broad number of compounds may result in a tedious procedure, if not evaluated with efficient methodologies. Classical one-factor-at-a-time approaches can be costly and time-consuming and multiple factorial effects cannot be identified and are not considered. To accelerate this phase, the use of DoE-based statistical methodologies is an adopted strategy in medium development and optimisation[18].

In a preliminary phase with many variables to be analysed, an efficient way to screen for the important factors with a limited number of experiments is the use of 2^k factorial designs. However, full factorial and 2^k fractional designs require a high experimental effort when there are many *k* factors to be analysed[19]. In this case, D-optimal Plackett–Burman designs (PBDs) become a useful alternative[20]. Still, PBDs are saturated designs developed to eliminate factors potentially having no or negligible effects over the responses. This means that only main effects are to be considered, which implies ignoring aliasing patterns due to higher order interactions[20]. Rejecting this possible scenario, which is very common in media optimisation[21,22], may guide upcoming experimentation to an incorrect decision. In this study, a folded-over matrix of the conventional PBD was implemented to improve the detection of factor interactions in the screening phase of a full DoE approach. The results obtained by the application of this technique were compared to the classical methodology.

After the identification of significant compounds influencing the response variable, the next step in a full DoE process is the optimisation of their concentrations through response surface methodologies[23,24]. The most widely used response surface methodologies include Central Composite Designs (CCDs) and Box-Behnken Designs (BBD). The selection among any of these methodologies is based on the number of k factors to be evaluated, the design space, and the optimality criteria[25]. For $k = 3$, Central Composite Designs require a higher number of experimental runs than the rest of the designs[19] and it has also been demonstrated that BBDs are slightly more efficient[26]. Accordingly, a BBD was selected for optimisation and the obtained polynomial equation was subjected to a best-fit iteration approach toward defining the smallest combination of significant compounds contributing to maximising the response.

The work presented here combines the sequential use of a folded-over PBD toward detecting possible synergies between different compounds in the screening phase followed by a best-fit BBD to optimise their concentrations. The optimum supplementation cocktail was validated and also tested in another CHO cell line stably expressing trastuzumab, an antitumoral monoclonal antibody against human breast cancer[27], in the same culture conditions in order to value the applicability of this compound combination.

Materials and Methods

Cell Line, Media, and Culture Conditions

The cell line used in the screening and optimisation steps in the present work is a serum-free suspension-adapted CHO-S cell line (ThermoFisher Scientific, Waltham, MA, USA). Also, a serum-free suspension-adapted CHO-S cell line expressing trastuzumab (Cobra Biologics AB, Keele, UK) was tested with the optimal condition obtained in this study. Two commercial serum-free media formulations for CHO-S were tested for cell growth. These include ProCHO5 (Lonza Biologics, Basel, Switzerland) and FreeStyle- CHO (ThermoFisher Scientific, Waltham, MA, USA). All formulations were supplemented with GlutaMAX (8 mM) (ThermoFisher Scientific, Waltham, MA, USA). Cell cultures were preadapted to each formulation prior to experimen- tation. Cells were routinely maintained and passed every 2–3 days in 125 mL disposable polycarbonate Erlenmeyer flasks (Corning, Steuben, NY, USA) in 20 mL of culture medium. Flasks were shaken at 130 rpm using an orbital shaker (Stuart, Stone, UK) placed in an incubator maintained at 37°C in a humidified atmosphere of 5% CO_2 in air.

Cell count and viability were determined using a Nucleo-counter NC-3000 (Chemometec, Allerod, Denmark) for eight days. The maximum specific growth rate, μmax (h^{-1}), and duplication time, $t1/2$ (h) were determined from the data corresponding to the exponential growth phase.

A YSI 2700 Select glucose/lactate analyser (YSI, Yellow Springs, OH, USA) was used to measure the concentrations of glucose and lactate in cell culture supernatants.

Toxicity Assays of the Compounds

Cell Titer 96 AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI, USA) was used to perform toxicity assays of all the compounds studied according to manufacturer instructions. Briefly, 100 µL of CHO-S cells was inoculated at 0.3 million cells/mL in a 96-well plate (Nalgene Nunc International, Rochester, NY, USA) together with 10 µL of the studied compound at different concentrations. After 48 h, 20 µL of Cell Titer 96 reagent was added to each well and then the 96-well plate was incubated for 1 h at 37°C and at 130 rpm orbital shaking. A calibration curve of known cell concentration (from 0 to 1.5×10^6 cells/mL) was performed in duplicate just before the analysis. The absorbance was measured in a Victor³ spectrophotometer (PerkinElmer, Waltham, MA, USA) at a wavelength of 490nm.

Compound Preparation

Non-animal-derived medium compounds used for media supplementation included three recombinant proteins: r-albumin (Merck Millipore, Kankakee, IL, USA), r-transferrin (Merck Millipore, Billerica, MA, USA), and r-insulin (Novo Nordisk Pharmatech A/S, Køge, Denmark); sodium selenite (selenium), synthetic cholesterol, fatty acids, (+)-α-tocopherol acetate, and tween 80 (Sigma, St. Louis, MO, USA). r-Transferrin solution was prepared from 20 mg/mL commercial stock in sterile PBS. r-Insulin, r-albumin, and selenium solutions were prepared from commercial powder stocks in PBS and subsequently 0.22 µm sterile filtered. Synthetic cholesterol and fatty acids solutions were prepared in PBS from 500*X* and 2000*X* commercial stocks, respectively. Tween 80 and (+)-α-tocopherol acetate were prepared from pure commercial stocks in sterile PBS to a concentration of 25 mg/mL and 2 mg/mL, respectively, both of them referred as 1000*X*.

Medium Optimisation Using DoE

The screening of the eight non-animal-derived medium compounds was performed with a folded-over PBD while BBD was chosen to optimise the concentration of the compounds with positive effect on cell growth. CHO-S cells were seeded at a cell density of 0.3×10^6 cells/mL in all DoE experiments and growth kinetics was followed every 24 h for eight days, thus, allowing the determination of the maximum viable cell concentration reached for each experimental condition tested.

Plackett–Burman Design

A two-time replicated 24-run folded-over PBD (Table 1) was used to identify the compounds with a significant effect on CHO-S cell growth and screen out negligible factors[20]. The eight compounds were screened at two levels: a low level (no additive) coded as −1 and a high level coded as +1. High levels for each factor were defined based on the toxicity experiments (Fig. 1) and on pre-existing knowledge from the literature[28]. To this purpose,

Exp no.	Selenium	r-Transferrin	r-albumin	r-Insulin	Tocopherol	Tween 80	Fatty Acids	Synthetic cholesterol	Response a	Response b
1	-1	+1	-1	+1	-1	-1	-1	-1	8.58	7.96
2	+1	+1	+1	-1	+1	-1	+1	+1	9.31	9.07
3	+1	-1	+1	-1	-1	-1	-1	+1	6.12	6.42
4	-1	-1	-1	+1	+1	+1	+1	+1	6.72	6.44
5	-1	-1	-1	-1	+1	+1	+1	+1	6.23	5.72
6	+1	+1	-1	+1	-1	+1	+1	-1	9.60	8.99
7	-1	-1	-1	-1	-1	-1	-1	-1	7.15	6.84
8	-1	+1	-1	-1	-1	-1	+1	+1	10.30	10.10
9	-1	+1	+1	-1	-1	+1	+1	-1	10.30	9.57
10	+1	-1	+1	-1	+1	+1	-1	-1	6.50	6.86
11	+1	-1	-1	-1	-1	+1	+1	+1	6.05	5.39
12	-1	+1	+1	+1	+1	+1	-1	+1	8.12	7.60
13	+1	+1	+1	+1	+1	-1	+1	-1	8.32	9.11
14	+1	+1	-1	-1	+1	-1	+1	-1	9.48	7.80
15	-1	-1	+1	+1	+1	+1	+1	-1	5.96	6.55
16	-1	-1	+1	+1	-1	-1	+1	-1	6.21	6.97
17	+1	-1	+1	+1	-1	-1	+1	+1	6.71	5.87
18	+1	-1	-1	+1	+1	-1	-1	+1	6.30	7.46
19	-1	-1	+1	-1	+1	-1	-1	-1	7.14	-
20	+1	+1	-1	-1	+1	+1	-1	-1	8.88	7.86
21	+1	+1	+1	+1	-1	+1	-1	+1	9.15	8.59
22	+1	-1	-1	+1	-1	+1	-1	-1	7.86	8.36
23	-1	+1	-1	+1	+1	-1	-1	+1	9.97	8.92
24	-1	+1	+1	-1	-1	+1	-1	+1	8.96	7.40

Factors	Coefficient	t-Value	p-Value
Constant	6.28	1.26	<0.01
r-Transferrin	0.77	0.89	0.38
r-Insulin	1.43	2.28	0.03
Tween 80	- 2.75	- 4.38	<0.01
r-Transferrin . r-Insulin	0.75	- 1.90	0.06
r-Transferrin . Tween 80	1.78	4.49	<0.01

Responses a and b are maximum viable cell concentration values from duplicate experiments in millions of cells/mL.

Table 1: Matrix design in coded levels, response and ANOVA analysis for the Plackett–Burman design with all factors and the best-fit model

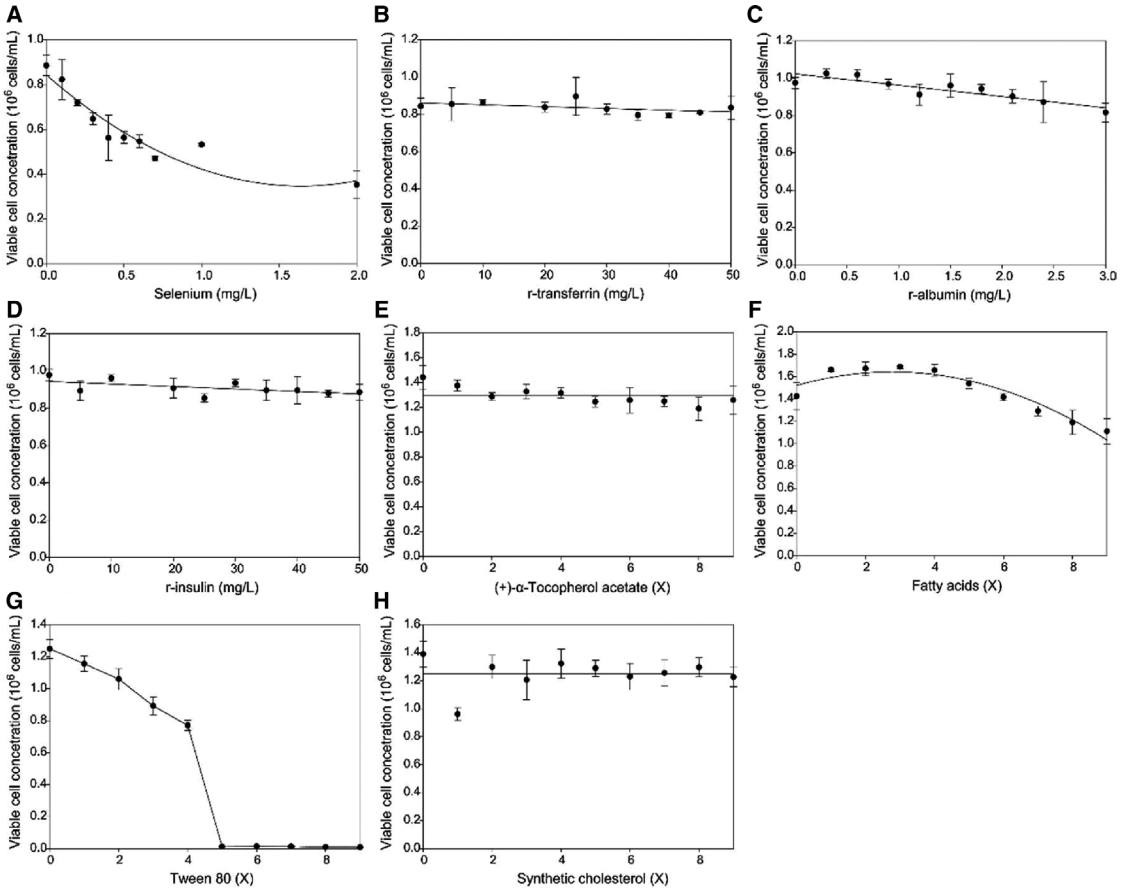

Figure 1: Toxicity assay curves for the different supplements in CHO-S cells; (A) selenium (mg/L); (B) r-transferrin (mg/L); (C) r-albumin

24-h amplified cells were split into 48 shake flasks with fresh medium and then the different compound combinations were added. Compound concentrations used for the PBD are provided in Supporting Information 1, whereas the experimental design matrix in coded values and maximum viable cell concentration (10^6 cells/mL) are shown in Table 1.

The effect of each experimental variable upon the measured response (viable cell concentration) was determined as the difference between the average responses at the high level (+1) and the average responses at the low level (−1), as shown in Equation 1:

$$E_{ij} = \frac{\sum Rj(+1)}{n/2} - \frac{\sum Rj(-1)}{n/2} \quad (1)$$

where *Eij* is the effect of the variable *i* on a response *j*, *Rj* is the measured response *j*, and n is the number of experimental runs. A positive value for *Eij* means that the variable *i* increases response *j* if added at the high level, and vice versa. Plackett-Burman experimental results were fitted to a first-order polynomial function with and without considering interactions by linear regression analysis as described below:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i \cdot X_i + \sum \sum_{i=1\ j>1}^{k\ k} \beta_{ij} \cdot X_i \cdot X_j + \varepsilon \quad (2)$$

Where *Y* is the response variable (cell density in million cells/mL), k is the number of factors, *β*0 is the model intercept term, *βi* the linear coefficient of the different parameters which correspond to one-half of the corresponding factor estimates, *βij* stands for the interaction coefficient, *Xi* and *Xj* are the studied compounds, and *ε* is the error associated to experimentation.

Box-Behnken Design

In order to define the optimal concentration for each factor selected in the screening phase, a Box-Behnken design was used. Compounds were screened at three equidistant levels: a low level coded as −1, a medium level coded as is the response variable (cell density in million cells/mL), k is the number of factors, *β*0 is the model inter- cept term, *βi* the linear coefficient of the different parameters 0, and a high level coded as +1, as indicated in Supporting Information 2. To do this, 24-h amplified cells were split into 30 shake flasks with fresh medium and the different compound combinations were added. Table 2 outlines the experimental design matrix in coded values, the response, and the statistical analysis of the method. The obtained results were fitted to a second-order polynomial equation by a linear regression analysis and subjected to an iteration-based refinement process (Equation (3)):

$$Y = \beta 0 + \sum_{i=1}^{k} \beta i \cdot Xi + \sum_{i=1}^{k} \beta ii \cdot X2_i + \sum_{i=1\ j>1}^{k\ k} \sum \beta ij \cdot Xi \cdot Xj + \varepsilon \quad (3)$$

where *Y* is the response variable (cell density in million cells/mL), *β* is the model intercept term, *β* the linear coefficient, *β* the quadratic coefficient, *βi* the interaction coefficient, *Xi* and *Xj* are the studied compounds, and *ε* is the error associated to experimentation. The model Equation (3) was used to predict the optimal concentrations of the selected compounds using the L-BFGS-B quasi-Newton algorithm implemented in the optimx package of the R software (R Development Core Team, Vienna, Austria). Three-dimensional plots were generated according to the equations toward facilitating model interpretation.

Exp no.	r-Transferrin	r-Insulin	Tween 80	Response a	Response b
1	-1	-1	0	8.17	7.82
2	1	-1	0	10.50	10.00
3	-1	1	0	8.71	7.84
4	1	1	0	7.80	7.95
5	-1	0	-1	5.80	6.14
6	1	0	-1	7.62	7.50
7	-1	0	1	5.48	5.56
8	1	0	1	5.06	4.36
9	0	-1	-1	7.40	8.80
10	0	1	-1	7.62	8.01
11	0	-1	1	8.19	6.68
12	0	1	1	7.83	7.12
13	0	0	0	8.09	8.12
13	0	0	0	7.74	7.67
13	0	0	0	7.53	7.83

Factors	Coefficient	t-Value	p-Value
Constant	7.83	35.53	<0.01
r-Transferrin	0.32	2.43	0.02
r-Insulin	- 0.29	- 2.17	0.04
Tween 80	- 0.54	- 3.98	<0.01
r-Transferrin . r-Insulin	- 0.66	- 2.52	<0.01
r-Transferrin . Tween 80	- 0.48	6.39	<0.01
r-Transferrin^2	- 0.50	- 7.01	<0.01
r-Insulin^2	1.27	- 3.48	<0.01
Tween 80^2	- 1.39	- 3.13	<0.01

All the variables were studied at three levels: a low level coded as −1, a medium level coded as 0, and a high level coded as +1. Responses a and b are maximum viable cell concentration values from duplicate experiments (runs 1–12) in millions of cells/mL. Run 13 was performed in triplicate because it was the centre point.

Table 2: Matrix design, response and ANOVA analysis for Box-Behnken experimental design of the different supplements: r-transferrin (X1), r-insulin (X2), tween 80 (X3)

Statistical Analysis

Statistical analyses of the models for PBD and BBD were performed using R Software with the *Frf2* and *car* packages. This license-free software was also used to develop the corresponding figures of the study. The quality of the regression of the model equations was evaluated by the coefficients R^2 and R^2. The validity of the equations was also checked by comparing the differences associated to experimental and pure error through the lack-of-fit (LOF) test (α = 0.05). The overall significance was determined with the ANOVA *F*-test (α = 0.05), whereas the significance of each coefficient was determined by the corresponding *t*-test (α = 0.05). Finally, normality of the calculated residuals was evaluated by means of the Shapiro–Wilk test (α = 0.05).

Trastuzumab Quantification

Trastuzumab concentration was determined by the commercial solid-phase enzyme-linked immunosorbent assay SHIKARI Q-TRAS (Matriks Biotechnology, Ankara, Turkey) according to manufacturer instructions. Briefly, 100 µL of Assay Buffer was added into each of the 96-well plate wells to be used. A total of 10 µL of each diluted sample and ready-to-use trastuzumab standards were added to the plate in triplicate. After 30 min of incubation at room temperature, the incubation solution was

discarded and the plate was washed three times. A total of 100 µL of peroxidase conjugate was added into each well. After 60 min of incubation at room temperature, the incubation solution was discarded and the plate was washed three times. Also, 100 µL of TMB substrate solution was added into each well and the plate was incubated in the dark for 10 min. The colorimetric substrate reaction was stopped by adding 100 µL of Stop solution and absorbance was measured at 450 nm in a Victor^3 spectrophotometer (Perkin Elmer).

Results

Selection of the medium for supplementation

ProCHO5 and FreeStyleCHO serum-free commercial media specific for CHO suspension culture were selected considering their use in industrial processes[29,30]. Cell growth of CHO-S cells was firstly characterised in these two media before starting with the sequential DoE approach. In both cases, cells maintained a high viability (> 90%) with a doubling time between 24 and 31 h at the exponential phase (Fig. 2A). A maximum viable cell concentration of 5.2 ± 1.0 × 10^6 cells/mL was attained between 96 and 120 h in FreeStyleCHO medium, whereas ProCHO5 reached 3.6 ± 0.9 × 10^6 cells/mL. When cell viability started to decrease, the availability of glucose was not limiting in ProCHO5 medium (> 1.5 g/L) while it was in FreeStyleCHO (Fig. 2B). However, adding a bolus feed of glucose to avoid this limitation did not improve cell growth (data not shown), hence indicating limitation by other factors in this medium. In respect to lactate production, it did not exceed 2 g/L in any media tested, which is typically considered the toxic concentration[31].

The two commercial media were then supplemented with a mixture of various compounds, here referred as MIX, in a concentration previously optimised for the HEK293SF-3F6 cell line[32]. This mixture included r-insulin (19.8 mg/L), r-transferrin (1.6 mg/L), (+)-α-tocopherol acetate (0.9*X*), tween 80 (0.9*X*), synthetic cholesterol (0.9*X*), and fatty acids (0.9*X*). As the concentration of the different compounds was not optimised for CHO cells, this experiment only consisted in a proof of concept to elucidate if the addition of these compounds to the media could improve maximum cell concentration. In all cases, cells maintained a high viability (> 90%) and the doubling time was reduced compared to non-supplemented conditions (Fig. 2A). Also, an increase in maximum cell concentration was evident upon supplementation, 1.3-fold in FreeStyleCHO (6.7 ± 0.7 × 10^6 cells/mL) and 1.5-fold in ProCHO5 (5.4 ± 0.4 × 10^6 cells/mL). In these conditions, lactate production did not exceed 2 g/L and it was consumed only in FreeStyleCHO medium since 120 h.

According to CHO cell behavior in each formulation, FreeStyleCHO was the medium selected for optimisation since cells reached a higher concentration with a reduced doubling time.

Toxicity assays for the compounds tested as supplements

Toxicity studies for each one of the individual compounds were performed in order to define the concentration ranges for the PBD.

Maximum viable cell concentrations after supplementation with varying concentrations of each compound are presented in Fig. 1. Fatty acids, tween 80, selenium, and to a lesser extent r-albumin were the compounds showing some cell growth

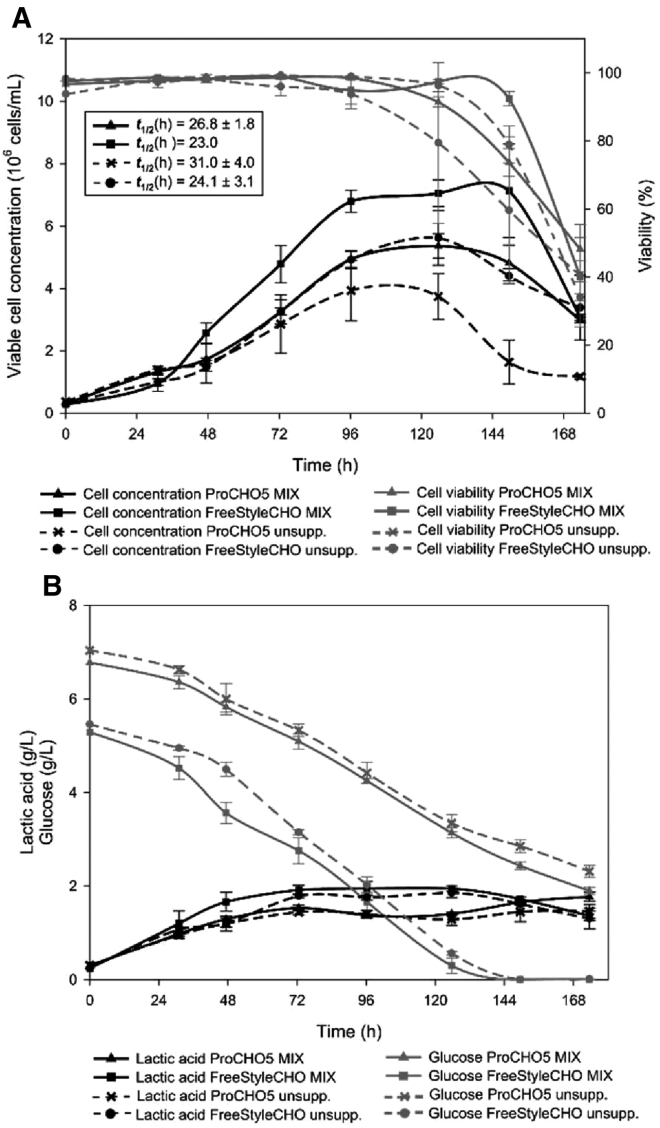

FIGURE 2: (A)Growth kinetics of CHO-S cells in batch culture in different culture media with and without MIX supplementation. Cells were seeded at 0.3 × 106 cells/mL in 125-mL flasks in their growing exponential phase. Cell density and viability of each culture were determined at every 24 h. (B) Lactic acid and glucose profiles in the different culture media with and without MIX supplementation. Mean values ± standard deviation of triplicate experiments are represented.

inhibition in the concentration ranges tested. These tests were used in combination with literature to properly set a suitable design space to perform the PBD (Supporting Information 1).

Compound screening using the folded-over PBD

A folded-over PBD of experiments was used as the screening methodology to find the significant compounds affecting cell growth. Two independent replications (n = 2) were performed for each experimental run in the matrix so as to calculate pure error.

A first analysis of the PBD_{12+12} results showed that linear regression without considering two-factor interactions could not accurately fit the experimental observations. The poor LOF test associated p-value of 0.004 indicated that only individual effects could not effectively explain data distribution. This suggested that effects firstly attributed to individual factors could be aliased with interactions between factors and that data from the PBD had to be analysed considering them. Linear regression combining main and interaction effects showed that the model was more efficient to fit the experimental data:

$$\text{Cell concentration } (10^6\text{cells/mL}) = 6.28 + 0.77 \cdot [r - \text{Transferrin}] + 1.42 \cdot [r - \text{Insulin}] - 2.75 \cdot [\text{Tween80}] - 0.75 \cdot [r - \text{Transferrin}] \cdot [r - \text{Insulin}] + 1.78 \cdot [r - \text{Transferrin}] \cdot [\text{Tween80}] \quad (4)$$

A non-significant LOF test p-value = 0.43, a R^2 coefficient 0.81 and a R^2 of 0.79 corroborated that the model containing individual effects and interactions fitted well. The statistical significance of the model was confirmed with an *F*-test associated p-value < 0.01 and each regression coefficient was analysed with the student's *t*-test (Table 1). The Shapiro–Wilk test of the residuals confirmed the normality assumption of the data since the null hypothesis was not rejected (p-value = 0.85). The predictive capability of the model was also assessed with the predicted error sum of squares value, which led to the calculation of a pred. R^2 coefficient of 0.75, very close to the R^2.

The comparison between the classical PBD_{12}, only based on individual effects, and the new approach here presented is shown in Fig. 3A–B, respectively. Main effect plots of the different compounds in both strategies include a colour-coding based on the student's *t*-test associated p-value. Student's *t*-test associated *p*-values lower than 0.05 (dark grey) imply that the compound is relevant whereas p-values > 0.05 (light grey) mean that there are no evidences to declare the factor as important. The classical approach showed r-transferrin as the only factor affecting cell growth, whereas the model containing interactions showed r-insulin and two two-way interactions comprising r-transferrin/r-insulin and r-transferrin/tween 80 as significant. The sole addition of tween 80 and the small interaction between r-transferrin and r-insulin proved to be deleterious for cell growth. However, the higher positive effects of adding r-insulin and the combination of r-transferrin and tween 80 counteracted the weak r-transferrin/r-insulin interaction and the negative effect of tween 80 by itself, respectively. Taking into account all these considerations, r-transferrin, r-insulin, and tween 80 were chosen for subsequent optimisation.

Optimisation of compound concentrations using the Box-Behnken design

An independently replicated (*n* = 2) three-factor Box-Behnken design was used to further optimise the concentrations of r-transferrin, r-insulin, and tween 80 in the culture medium. In this phase, the range of r-insulin concentrations was reduced since no improvement in cell growth was observed beyond 2 mg/L.

Maximum viable cell concentrations for each experimental run were fitted to a second-order polynomial as described in Equation 3. The obtained equation was subjected to a refinement process to find the best fitting with the lowest number of parameters:

$$\text{Cell concentration} = 7.90 + 0.27 \cdot [r - \text{Transferrin}] - 0.29 \cdot [r - \text{Insulin}] - 0.47 \cdot [\text{Tween80}] - 0.66 \cdot [r - \text{Transferrin}] \cdot [r - \text{Insulin}] - 0.48 \cdot [r - \text{Transferrin}] \cdot [\text{Tween80}] - 0.59 \cdot [r - \text{Transferrin}]^2 + 1.30 \cdot [r - \text{Insulin}]^2 - 1.49 \cdot [\text{Tween80}]^2 \quad (5)$$

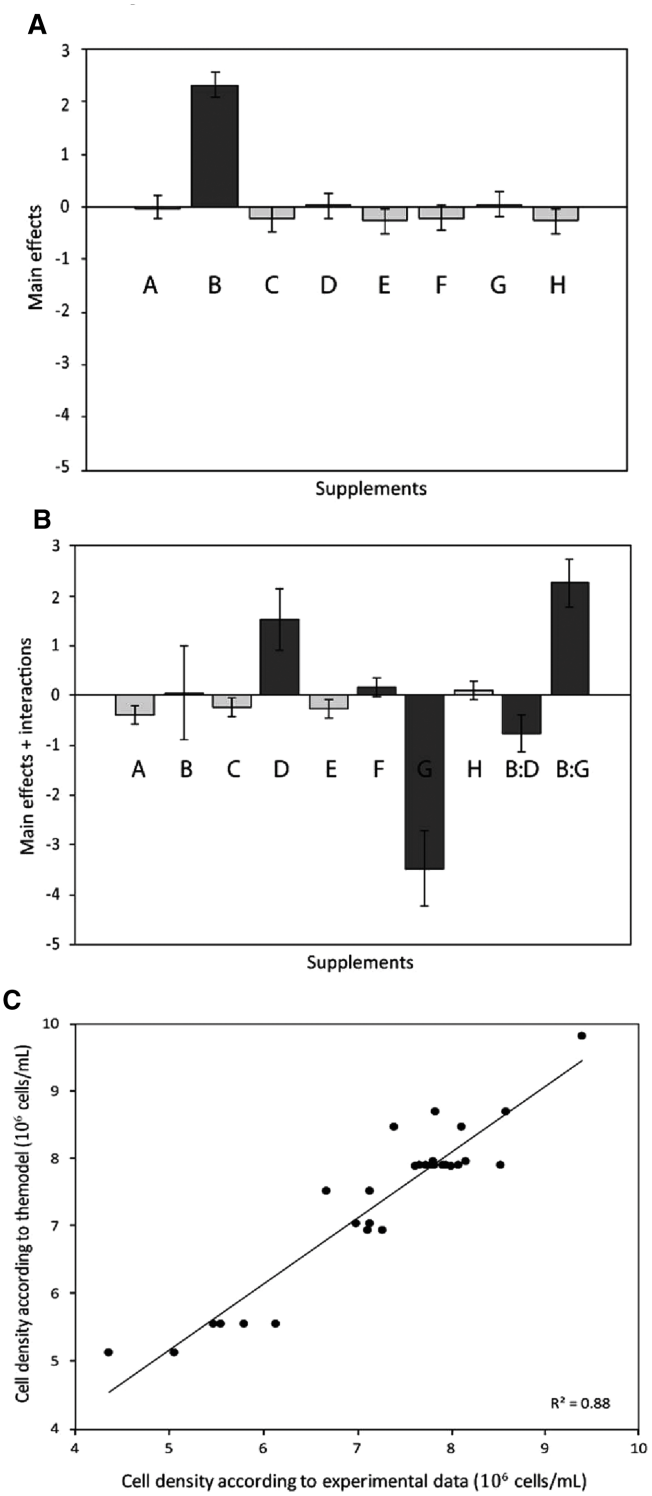

FIGURE 3: (A) Main effects plot of the PBD only considering main effects of non-animal-derived supplements on CHO-S cells in FreeStyleCHO medium, where: A, selenium (mg/L); B, r-transferrin (mg/L); C, r-albumin (mg/L); D, r-insulin (mg/L); E, (+)-α-Tocopherol acetate (X); F, fatty acids (X); G, Tween 80 (X); H, synthetic cholesterol (X). Dark grey bars represent significant factors (p-value < 0.05) whereas the light grey ones mean non-relevant factors (p-value > 0.05). The response variable is maximum viable cell concentration (106 cells/mL). (B) Main effects plot of the PBD considering main effects with relevant interactions of the different compounds. (C) Correlation between the experimental data and Box-Behnken model predicted cell densities. Mean values ± standard deviation of duplicate experiments are represented.

Regression analysis showed that the model was adequate with a coefficient R^2 of 0.88, which suggested that 88% of the variability in the data was explained by the model Equation (5) (Fig. 3C). The R^2 value of 0.83 and the *pred.* R^2 of 0.75 further corroborated model adequacy.

The statistical significance of the model was confirmed with an *F*-test associated *p*-value < 0.01. The significance of each regression coefficient of the best-fit model was analysed by Student's *t*-test and is shown in Table 2. In all cases, Student's *t*-test associated *p*-values < 0.05 and, thus, all terms were considered significant. The study of the residuals by means of the Shapiro–Wilk test confirmed the normality assumption of the data (*p*-value = 0.23). In conclusion, the ANOVA analysis of the model Equation 5 was satisfactory for the experimental data obtained with the BBD and the model could be used to navigate the design space.

Response surface analysis of the BBD

Three-dimensional plots were constructed for visual analysis of the response trend regarding different combinations of r-insulin, r-transferrin, and tween 80 (Fig. 4).

The multifactorial nature of this optimisation could be observed in the two optima identified by solving the second-order polynomial equation (5) (Supporting Information 3). These two different compound combinations provided the highest levels of cell concentration, one with a high level of r-transferrin and another with the highest level of r-insulin and a moderate level of r-transferrin (Fig. 4A-C). Of note, the negative interaction between r-transferrin and r-insulin previously encountered in the PBD was also present in the BBD (Fig. 4E). As r-transferrin concentration increased, the optimum condition moved from higher to lower r-insulin concentrations (Fig. 4D-F). Tween 80 levels were similar in both optima and around the middle of the evaluated concentration range (Fig. 4G-I).

The optimum with r-transferrin reaching very high levels (57 mg/L) was considered difficult to be adopted in practical terms due to the high cost of r-transferrin. Therefore, the optimum condition making a higher significance was the one with 21.3 mg/L of r-transferrin, 2 mg/L of r-insulin, and 1.8*X* of tween 80. Under these conditions, the predicted maximum viable cell concentration was 9 ± 1.1 × 10^6 cells/mL.

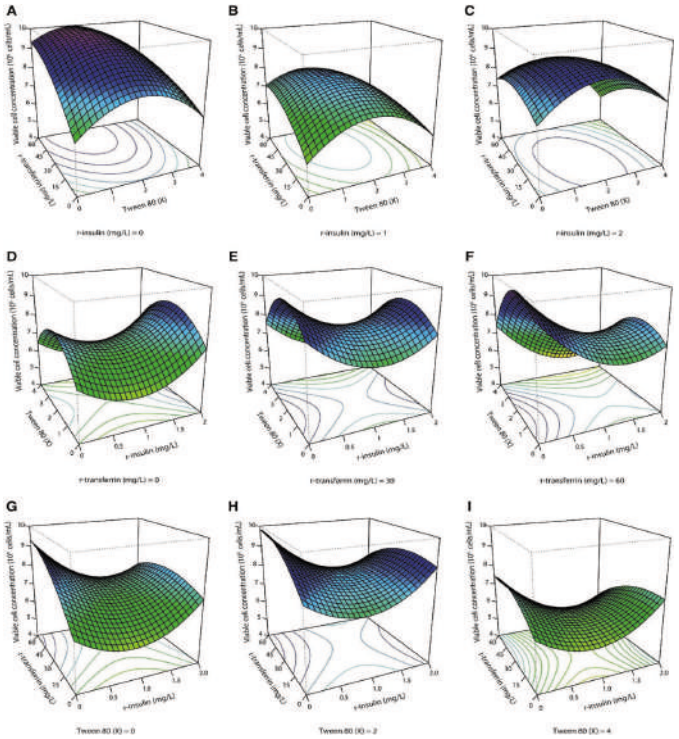

FIGURE 4: Response surface graphs based on Box-Behnken experimental results (A–I). Maximum viable cell concentration in cell culture as a function of the concentrations of (A–C) r-transferrin versus tween 80; (D–F) tween 80 versus r-insulin; and (G–I) r-transferrin versus r-insulin. The graphs were constructed depicting two variables at a time while keeping the third one at a fixed level.

Validation of the model

A validation experiment was carried out to corroborate the optimal supplementation conditions predicted by the second order polynomial. A maximum viable cell concentration of 10 ± 0.6 × 10^6 cells/mL (*n* = 3) was attained under these conditions (Fig. 5A), close to the model prediction (9 ± 1.1 × 10^6 cells/mL), confirming model accuracy. Of note, a 1.5-fold improvement was obtained for CHO-S cells growing in the optimum condition compared to non-supplemented FreeStyleCHO medium (6.6 ± 0.4 × 10^6 cells/mL). A better adaptation to the supplemented medium could also be observed since cell-doubling time was significantly shortened from 23 to 20 h (Student's t test associated p-value = 0.048).

Supplementation of a trastuzumab expressing CHO-S cell line

In order to generalise the results obtained with the application of this new DoE approach, a CHO-S cell line expressing trastuzumab

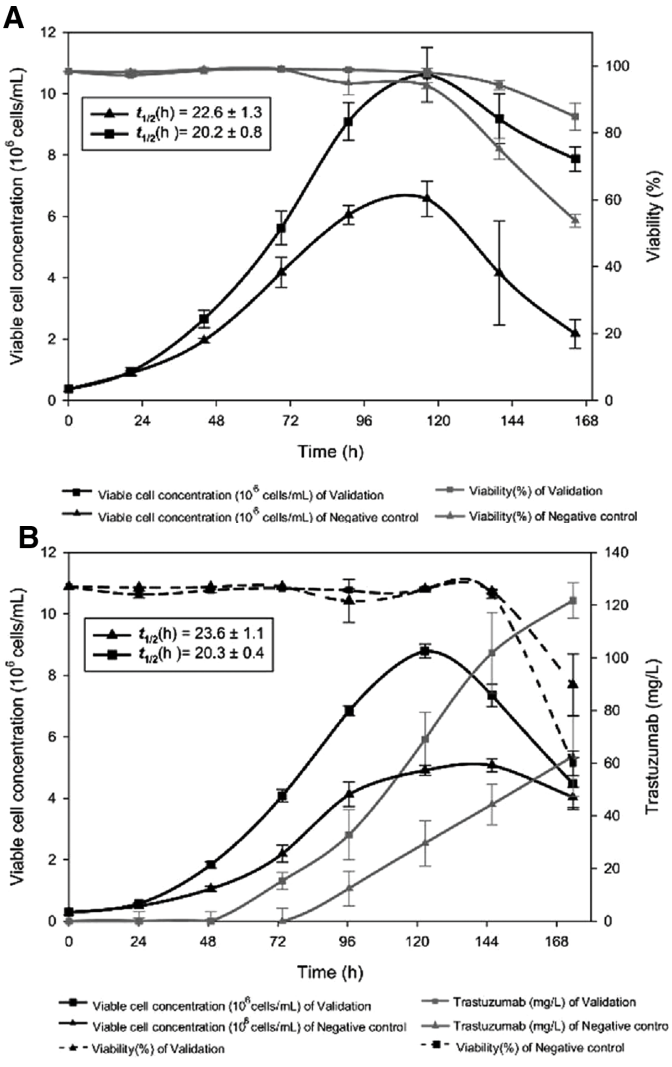

FIGURE 5: (A–B) Model validation. (A) Unsupplemented (Negative control) and supplemented CHO-S cell line in FreeStyleCHO medium with the optimal levels of r-transferrin, r-insulin and tween 80. (B) Non-supplemented and supplemented CHO-S cells expressing transtuzumab in FreeStyleCHO medium with the optimal levels of r-transferrin, r-insulin and tween 80. Cell density and viability were determined daily. Mean values ± standard deviation of triplicate experiments are represented.

was used in the same culture conditions. In this context, 121.8 ± 6.7 mg/L was obtained, which represented a two-fold increase when compared to non-supplemented FreeStyleCHO medium (62.5 ± 15.3 mg/L) as observed in Fig. 5B. A maximum cell concentration of 8.8 ± 0.2 × 10^6 cells/mL was obtained upon supplementation, a 1.7-fold increase when compared to the non-supplemented medium (5.1 ± 0.3 × 10^6 cells/mL). As for the non-expressing CHO-S cell line, the doubling time significantly decreased from 24 to 20 h in supplemented conditions (student's t test associated p-value = 0.008).

Discussion

In this study, a powerful approach for compound screening based on the use of a folding-over PBD was successfully used with the objective to improve cell growth and production in CHO-S cells. The use of this methodology in a preliminary phase of a full DoE approach enabled the detection of some compounds that were negligible when considered individually but they were not when used in combination.

Considering the increasing pressure on cell culture-based processes in terms of production, media optimisation is of interest especially if a systematic approach is followed in its definition[9]. Media supplementation is more affordable for the majority of cell culture users compared to the development of an in-house formulation. The latter normally includes a lot of different compounds and the effort required in time and resources is very high. Commercial media is a good alternative to full media development, however, their composition is not known and they are developed for a scope of cell lines. Therefore, there is an opportunity for supplementation to substantially improve the yields in cell concentration and product titre by relatively modest efforts.

A general approach toward media optimisation is to first use a screening design to select the relevant compounds showing a positive effect on cell culture[33]. In this sense, the resolution III PBD is an accepted methodology that fits to this purpose. However, most publications related to media optimisation do not explore further in the nature of the effects caused by each of the compounds[34,35]. Aliasing arising from the synergies between the different compounds with the main individual effects may compromise the interpretation of the results, which is often not considered in the early steps of media supplementation[36]. This is a critical decision, which may guide the next steps in experimentation to an incorrect decision (i.e. optimisation designs). The classical PBD contains an alias structure between main effects and two-factor interactions that can be resolved by folding-over the design. To do this, the Hadamard's matrix of the PBD is increased with the opposite combinations of the initial runs.

Alternative designs, such as the 2^{8-4} or the 2^{8-3} fractional factorials either cannot untangle *IV* two-factor *V* interactions or they require more experimental runs compared to the 24-run folded-over PBD, respectively. The key to folding over is choosing all the individual factors considered in the PBD_{12+12} and performing a linear regression. If the LOF test associated p-value is significant, there is evidence that interactions are relevant and need to be considered in the model[37]. The determination of these interactions is based on a trial and error process considering the effect heredity, hierarchy, and sparsity principles[38]. The first rule states that it is most probable for an interaction to be active if at least one of the factors involved has an active main effect. The second and third principles assume that lower-order is more important than higher-order effects and that the number of relatively important factors is small, respectively[39]. The comparison of the folded PBD_{12+12} to the classical PBD_{12} revealed the aliasing pattern between r-transferrin and the combination of r-transferrin with r-insulin and r-transferrin with tween 80, which could not be detected by the classical PBD approach.

Using the folded-over PBD in combination with a BBD defined an optimum supplementation cocktail of 1.8X of tween 80, 21.3 and 2 mg/L of r-transferrin and r-insulin, respectively. Under these conditions, a maximum viable cell concentration of 10 × 10^6 cells/mL was obtained in batch culture, representing a 1.5-fold increase in maximum cell concentration compared to the non-supplemented medium. The optimum condition was also used in a CHO-S cell line stably expressing trastuzumab with a 1.7-fold improvement of cell growth and 2-fold increase in final antibody titre. A comparison of the specific antibody productivity (q_p) revealed that the improvement in trastuzumab concentration was a consequence of a higher cell concentration and not of an increase in qp. Indeed, the comparison of both specific antibody productivities based on trastuzumab titre and the integral of viable cell concentration resulted in 1.3 ± 0.3 × 10^{-10} for the supplemented medium and 1.2 ± 0.4 × 10^{-10} mg/(cell · h) for the non-supplemented one. Student's t-test associated p-value of 0.91 indicated that there were no significant differences between both conditions in terms of specific antibody production. Of note, efficient supplementation of commercial media for a specific cell line is paramount toward maximising the final protein titre and reducing the production costs.

Insulin and transferrin in combination with other compounds have been reported to have a variety of positive effects on mammalian cell culture. For example, combining them with selenium (ITS) in CHO-DG44 cells improved cell growth by 35%[40]. The only combination of insulin and transferrin had also proven to increase 18% TNFR-Fc production in a GS-CHO cell line[41] and prourokinase production in 11G-S CHO cells[42]. Tween 80 has recently been demonstrated to have a beneficial effect on mAb production in CHO-K1 and CHO DUX-B11[13]. The authors mention that the interest in using tween 80 relies on its capacity to extend the stability of media compounds in solution for a longer period of time, which spans their availability in the culture medium. In this regard, the combination of tween 80 with r-insulin and r-transferrin is shown as an interesting supplementation cocktail for CHO-S-derived cell lines compared to reported insulin and transferrin combinations, in which lower growth and production yields are achieved[40,41].

In conclusion, an advantageous approach for compound screening and medium supplementation was successfully developed in this work. This methodology deepened in the synergies between compounds at early screening stages and highlighted the importance of considering these factor interactions in later experiments.

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Conflicts of Interest
The authors declare no conflicts of interest.

Ethical Approval
This article does not contain any studies with human participants or animals performed by any of the authors.

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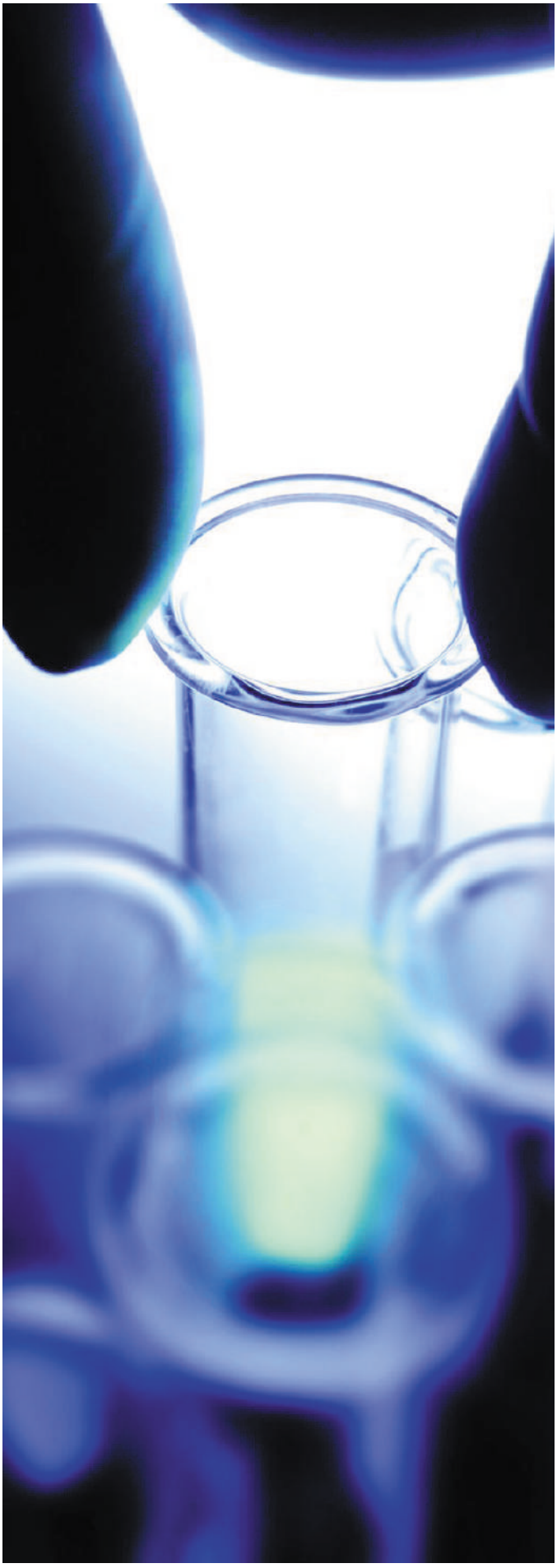

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PEER REVIEWED

Importance of the Quality System: Production, Quality Control & Quality Assurance

An effective quality system is the most important aspect of manufacturing any biological or pharmaceutical product. If the system does not ensure that Production, Quality Control and Quality Assurance are actively communicating, a potential system failure could occur. Therefore, it is imperative that each of the three areas understand the importance of the other in order to prevent failures in the quality system.

Introduction

There are many important factors that can affect a quality system; however, Production, Quality Control and Quality Assurance have responsibilities that could greatly impact the process. Each area is critical to ensure the processing of the product effectively moves from one area to the next. Abiding by the procedures and executing aseptic techniques guarantee that the quality system will continue to remain intact. The quality system will be successful if and only if each area understands the importance of its individual role. If this is achieved, the product will be completed with integrity, accuracy and the highest degree of quality.

Production

The production process for any biologic or pharmaceutical product is to formulate, blend, and fill to specifications in order to meet the acceptance criteria. As a result, Production has an important role in beginning the process for the product. It is crucial that Production follows all standard operation procedures (SOPs) in their entirety and reflect what is needed for the success of the process. The production process is loaded with various tasks in addition to formulating, blending and filling. An example of this is the washing of glassware and disinfecting of critical areas. It is imperative that there be a clear understanding of Production's contribution to the quality of the product.

Gowning, personal protective equipment (PPE) and risk assessment procedures are key when it comes to the process of producing a good quality product. It doesn't matter whether the personnel are in the lab or cleanroom, gowning/PPE are vital because they protect the individuals from any harmful materials and simultaneously prevent contamination. Production personnel are continuously interacting with the product from the beginning of the process when it comes into the system (in its raw material form) until it is a part of the final product. Production personnel must always ensure that they have had proper training for gowning to protect the product within the quality system.

Production personnel should always certify that all instruments, glassware or needed items are cleaned, disinfected and prepared prior to formulating. Calibration is necessary to guarantee that the data reported by the instrumentations are accurate. Ineffectively cleaning glassware and/or lack of calibration can lead to product contamination. As a result, it would be difficult to determine even after further investigation if production personnel followed the SOPs that were set. Though this seems minor, failure to do so could potentially compromise the product and affects the final quality of the product.

Quality Control (QC)

Quality Control (QC) like Production, is essential to the quality of the product. QC gives a deeper understanding of what is occurring and how the product is developing through the production process. QC is responsible for determining the sterility, stability and effectiveness, and interpreting the results to Production. Without QC being involved in the process, Production would not be able to identify the accuracy of the process.

From the beginning of the production process to the end, QC is involved in the process development. Depending upon the manufacturer, QC may have to perform incoming tests to confirm that the information specified on the certificate of analysis aligns with the material received from the supplier. Confirmation of the material received begins the process of quality checks to reduce the chances of issues with the finished product. Following the receipt of raw materials for creating the product, QC tests the incoming materials to determine its acceptability for use so that production can incorporate the material into the formulation.

In-process and final product testing are important tasks for QC. In-process testing is done in order to perform a thorough check of how well the production process is progressing. Final product testing is also done to guarantee that the product has met all the requirements for release. Final product testing provides the last needed information about the product to ensure the safety and effectiveness that is necessary to determine that the product was under the required control.

If there are any issues that arise from the quality checks by QC, Production can be notified to cease the current process. This strategy assists Production in determining where in that cycle the quality deviated from specifications. With quality checks in place, the operation has a clearer picture of how it is functioning. If the process passed prior to the current quality check, then that part of the process is acceptable. If at the current quality check the results fail, there is a greater possibility that the issue occurred in that part of the process and not prior quality checks. The failure could have been the result of a newly added material.

Prior to assuming the occurrence or failure took place in Production, it is important to confirm whether the error occurred during testing. Both a thorough review of the data collected, and an evaluation of the technician's own technique, must be done. If the possible error occurred in QC, a retest of the product would be necessary to properly rule out human error.

Interaction Between Production and Quality Control

Production and Quality Control (QC) have many areas of overlap during the process of developing a product. Environmental Monitoring (EM) provides an overview of the environment that the product will be exposed to that may have an adverse effect on the product. The purpose of EM is to demonstrate the control of both viable and non-viable particles in critical areas like the cleanroom. To keep the product sterile and free from contaminations, QC must keep track of the number of particles and colonies that form.

Prior to interacting with the product, each active person in these areas must be stamped with RODAC plates to ensure proper gowning and that they are not introducing viable or non-viable particles into the production area. Not only is EM of the personnel important, but also the monitoring of the particles in the air or surfaces in the proximity of the product being manufactured. QC will generate EM data of the activity from particles by using air samplers, setting plates and/or contact plates. Without this interaction of QC and Production, the product may be exposed to contaminations. Any organism recovered should be identified to give more insight on what is occurring during product manufacture. This will authorise production personnel to implement procedures to eliminate or prevent any potential contamination.

Quality Assurance (QA)
Quality Assurance personnel are the police officers of the quality system. These individuals are the enforcers of controlled documentation, reviews and quality checks to guarantee a high-quality product. It is the due diligence of Quality Assurance to confirm that the acceptable standards are met. QA oversees the entire production process since these individuals are reviewers of all the data generated from both production and QC. QA must thoroughly understand the process to be able to approve the documents needed prior to sending the information to the respective regulator for release. The documents explain in detail what actions were performed with results for the product. Each section is reviewed in detail and anything that deviates from the criteria should be investigated to determine what has caused these actions to arise.

QA ensures that every area perform and adhere to all approved SOPs which are required for a good quality system. QA maintains employee training records and makes certain that the personnel accomplish any trainings that are outstanding. It is imperative that all personnel are fully trained and are current with their training. Their actions must reflect what is stated in the training documents.

As the representative of the quality system, QA handles the interactions with regulators. During audits from respective regulators, QA personnel act as the spokespersons of the quality system for the production facility. These individuals aid the inspection of necessary documents and information which could be provided to the regulator. Since QA controls, structures and facilitates the quality system, they are able to interpret information to better assist regulators for a successful audit.

Quality Assurance Interactions with Production and Quality Control
Since quality assurance (QA) personnel are the enforcers of the quality system, it is important for these individuals to manage the process of production and quality control (QC). This is critical when there are any deviations or if the product is out of specifications. When there are situations where the product deviates or does not conform to the processes, then deviation reports must be generated. This may cause a halt in the production operations. When deviations occur, QA must have knowledge of what has taken place in an established production. At this stage, an investigation must be initiated. To get a complete understanding, QA should review the results generated by QC and understand where in the production process the occurrence took place. With these tools, QA can gather all supporting evidence like information from Production, data from QC and its own understanding of the process to be able to draw a clear conclusion of what happened. This will help determine if the deviation affected the product in any way that would deem it unacceptable.

After gathering all supporting documents and evidence, QA may specify the corrective action and preventive actions (CAPA) in an attempt to eliminate the deviation from occurring again. CAPAs are important to have because it is the resolution to the issue and confirms that the situation was handled properly as soon as the problem was identified. This gives regulators the assurance that the manufacturer took the proper actions to correct an issue that was presented. All three sections must work together so QA can generate a complete and valuable deviation report.

Lack of Communication Leading to Failures
Communication is the most crucial aspect of the quality system. Within each area of the quality system, personnel should communicate with one another to assist each other if anyone is out of compliance or is not properly executing what is stated in the SOP. It is everyone's responsibility to make sure and be held accountable for the product which reflects the manufacturer. For example, if QC does not relay that data generated from the tests to reflect that process is faulty and the problem is not noticed by Production, then the final documents will be submitted with faulty information to QA. This failure, however, should be caught by QA because they are knowledgeable on how to interpret the data from Production and QC. In this instance, they would have the authority to prevent the product from being released by the facility. If such a failure is not caught by QA, this poor-quality product could possibly be released to the public which would then directly become affected. In addition, it could lead to major investigations by regulators and ultimately result in a product recall. Regulators would then have the power to pull the manufacturer's licence to produce the product. For this reason, all three areas have an obligation to communicate with one another to prevent detrimental failures in the system.

Conclusion
The way to keep the quality system intact is for each area to have a thorough understanding of its role and impact to the system. Each person involved in the quality system must assist and converse with one another to ensure that the product produced will be successful. It takes every area to yield a high-quality product. In conclusion, all areas must continue to work together as a quality system to guarantee the safety of the product for release to the public.

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This article is a general overview of three areas involved in the quality system. For more in depth information about various quality management systems, please refer to the items below:

1. CFR Part 820: Quality System Regulations
2. ISO9001:2015 Quality Management System
3. ICH Q10: Pharmaceutical Quality Management

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Rapid Host Cell Protein (HCP) Detection with Advanced Trapped Ion Mobility Spectrometry (TIMS) Technology

The biopharmaceutical field is rapidly expanding, with biotherapeutic analysis and bioprocess development seeing particularly significant growth in recent years. The market has a projected growth of 8.59% CAGR from 2019–2024[1], which will continue to help the industry address the unmet medical needs for the world's most challenging diseases.

The percentage of large molecules in the pharmaceutical pipeline rose from 30% in 2010 to 42% in 2017[2] and there are nine Food and Drug Administration (FDA) approved biosimilars on the market, five of which are from 2017. There is, therefore, an ever-increasing requirement for analytical methods with rigorous process-related impurity testing, to monitor these contaminants during manufacture and prior to product release.

During biotherapeutic manufacturing, process-related impurities derived from the host organism can contaminate drug products. For example, during the expression of a recombinant protein drug, host cell systems can express numerous endogenous proteins, known as host cell proteins (HCPs). These contaminants may significantly affect drug efficacy and sometimes cause immunogenicity, making their removal highly important to ensure drug safety and purity. Biotherapeutics, such as monoclonal antibodies (mAbs), are purified using chromatographic techniques designed to remove residual HCP, DNA and viruses, as well as product-related impurities, such as fragments and aggregates. However, low-level HCPs often remain after purification, so the detection and quantification of these residual impurities is critical for biopharmaceutical companies to comply with regulatory guidelines.

Impact of Residual HCPs

There are several ways in which biotherapeutics contaminated with residual HCPs could compromise patient safety. The drug's efficacy and toxicity can be affected, and the therapeutic window in which the drug acts can be altered. Immunogenicity is the primary concern, where the HCP can invoke an unwanted immune response and cause damage to the patient, even at trace levels. For example, the clinical trial of IXinity (formerly IB1001) for the treatment of haemophiliacs was suspended in 2012, due to the development of antibodies to the drug in 26% of patients[3].

The stability of biotherapeutics may also be compromised by the presence of residual HCPs. They can contain enzymes, such as oxidases and lipases, which gradually break down the therapeutic protein or excipient, affecting the stability of the product over time. HCPs are also capable of mimicking the action of therapeutic proteins in assays, possibly resulting in the misformulation of the product outside the therapeutic window [4].

Meeting Regulations

The amount of residual HCP in a drug product is generally considered a critical quality attribute (CQA), due to its ability to impact product safety and efficacy. The International Conference on Harmonisation (ICH) Q6B requires the detection and quantification of process-related impurities, such as cell substrates (including HCPs and host cell DNA), as well as cell culture or downstream processing[5]. It also stipulates that, where possible, the biological activity of impurities should be evaluated.

HCPs are measured in parts per million (ppm), often expressed as nanograms per milligram of the intended recombinant protein. The general target for analysis is to ensure identification of HCPs at least down to the 100 ppm level. However, many contaminating enzymes in the HCP mixture can still be active at levels below 100 ppm, so highly sensitive detection technologies are, therefore, desirable for biopharmaceutical manufacturing organisations.

Impurity Testing Technologies

Comprehensive HCP analysis allows biopharmaceutical manufacturers to ensure quality control between batches, and facilitates comparisons between biologic drugs and biosimilars. The gold standard technique for HCP analysis is the enzyme-linked immunosorbent assay (ELISA), which is a commonly-used immunoassay for measuring antibodies, antigens, proteins and glycoproteins in biological samples. Despite its high throughput, sensitivity and selectivity capabilities, the ELISA can overlook weak and non-immunoreactive HCPs, which still carry the potential to impact drug safety and efficacy. It is also process-specific, meaning a new assay is required after each process change, which may take several months.

Many biopharmaceutical companies are now looking towards orthogonal methods, such as mass spectrometry (MS), as an alternative or complementary technique for HCP analysis. MS can rapidly monitor and identify multiple protein analytes in the same sample, and detect very low amounts of HCP in a non-targeted manner. This is crucial, considering that even very low-level HCPs can cause immunogenicity and impact drug quality. Whereas an ELISA can only measure the total HCP, MS can provide detailed information on the level of each individual HCP. Advanced MS techniques not only monitor, but characterise several impurities using a single method, bringing the high discriminatory power needed to separate impurities, while providing high sensitivity to detect and quantify low-level HCPs.

Analysing HCPs with MS

Although MS is gaining popularity as a powerful technique, there are still some limitations to overcome. A mass spectrometer with up to six orders of magnitude dynamic range is required to directly detect 1 to 100 ppm of HCPs in the sample, which is out of the range of many current instruments[6]. One way of overcoming the issue of dynamic range for HCP characterisation is to use ion mobility. Some modern instruments use trapped

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ion mobility spectrometry (TIMS) quadrupole time-of-flight (QTOF) MS to achieve high confidence in identification and deep coverage, without compromising speed.

TIMS is a separation technique in gas phase, which resolves sample complexity with an added dimension of separation, in addition to high performance liquid chromatography (HPLC) and MS. This method increases peak capacity and confidence in compound characterisation and, combined with parallel accumulation serial fragmentation (PASEF) technology, enables high sequencing speeds while generating high-quality spectra. The high resolving power of TIMS MS makes it useful for the analysis of complex samples, such as those of biological origin.

PASEF scans implemented on TIMS QTOF MS/MS can combine fast acquisition speeds (>100 Hz MS/MS) with high resolution detectors, for high resolution quantifiable data from a complex sample, in a single run. When coupled with an advanced HPLC system, TIMS QTOF MS/MS with PASEF can detect HCPs using a gradient as short as 21 minutes. PASEF enables almost 100% duty cycle, for maximum use of all ions and therefore superior sensitivity.

There are two approaches to HCP analysis by MS:

1. HCP discovery

This method is useful in early process development, where critical problems arise in production or stability testing. HCP discovery uses longer runs for increased depth of analysis, typically two hours or more. Quantitation is not always necessary, as the primary concern is identifying all the proteins in the mixture. Maximum depth of coverage can be achieved, to identify low-level proteases/lipases that can affect stability, as well as other problematic proteins. Hundreds of peptides can be identified in a single run (Figure 1).

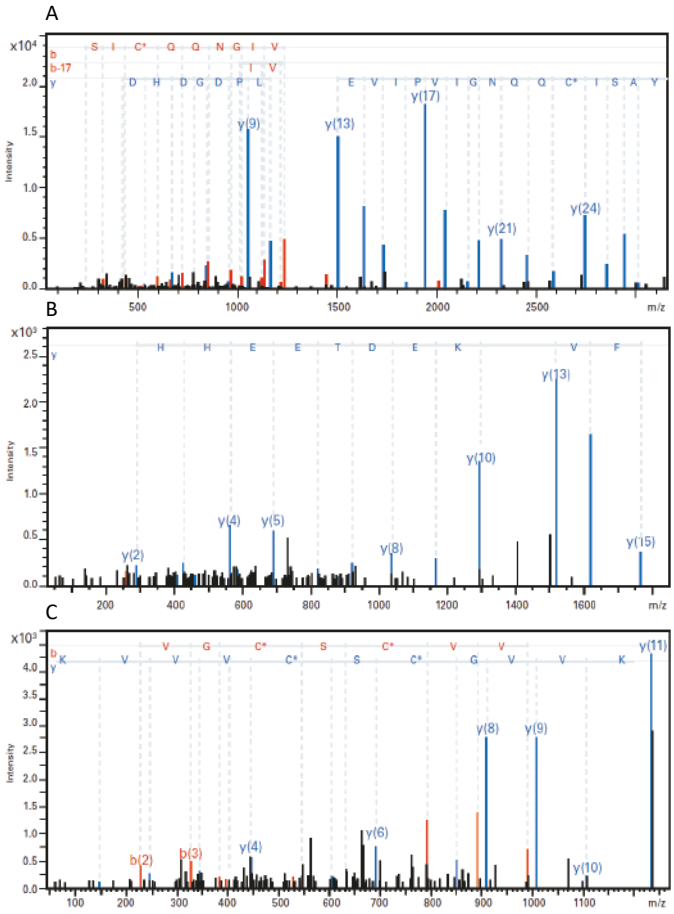

Figure 1: High-quality TIMS QTOF MS/MS with PASEF spectra (timsTOF Pro, Bruker Daltonics), featuring high sequencing speeds of > 100 Hz and sensitive detection of low abundant proteins (A) Fructose-biphosphate adolase A, (B) Heterogeneous nuclear ribonucleoprotein A1 and (C) 40S ribosomal protein S12.

For a 1.5 µg NISTmAb tryptic digest, nanoLC provides a 210-minute gradient for deep coverage of HCPs. Sample preparation can either be a standard tryptic digest of the entire mixture (220 HCPs identified), or a native tryptic digest (using protocol from[6]), where the mAb is left in its native structure, which provides a better dynamic range (280 HCPs identified).

2. Fast HCP screening

The method provides rapid analysis of downstream processes, with fast runs and high throughput. Quantitation is often necessary. Faster analysis is advantageous for more routine applications, but coverage of 50+ HCPs is still attainable at 1% false discovery rate (FDR) using a 21-minute gradient (Figure 2).

Description	Coverage	#Peptides	#Unique	Avg. Mass
Fructose-bisphosphate aldolase A OS=Mus m...	74%	35	30	39356
Glucose-6-phosphate isomerase OS=Mus mus...	46%	26	26	62767
Fructose-bisphosphate aldolase C OS=Mus m...	53%	18	13	39395
Semaphorin-4B OS=Mus musculus GN=Sema...	10%	7	7	91392
Ig gamma-3 chain C region OS=Mus musculu...	7%	7	6	43929
Protein ABHD11 OS=Mus musculus GN=Abhd1...	35%	6	6	33561
Protein disulfide-isomerase A6 OS=Mus musc...	13%	4	4	48100
Low affinity immunoglobulin gamma Fc region...	11%	4	4	36695
Polypeptide N-acetylgalactosaminyltransferase...	7%	3	3	71537
Syntaxin-12 OS=Mus musculus GN=Stx12 PE=...	18%	3	3	31195
NSFL1 cofactor p47 OS=Mus musculus GN=Ns...	16%	3	3	40710
Fumarate hydratase, mitochondrial OS=Mus ...	9%	3	3	54357
Methionine--tRNA ligase, cytoplasmic OS=Mus...	5%	3	3	101431
Nucleoside diphosphate kinase B OS=Mus mu...	26%	3	3	17363
Adenylate kinase 2, mitochondrial OS=Mus m...	21%	3	3	26469

Figure 2: Host cell proteins (HCPs) identified in 21 minutes with three or more peptides. Data were acquired on the timsTOF Pro (Bruker Daltonics) applying a 0.5 sec cycle consisting of one TIMS MS scan and four PASEF MS/MS scans.

Both methods provide unbiased analysis of proteins, accurate mass measurement and, because of the high resolution of both MS and MS/MS, isotopic fidelity. They also provide collisional cross-section (CCS) values for a new dimension of confidence. These are physical parameters of peptides, often more reproducible than LC retention times. Novel MS instrumentation, such as the timsTOF Pro with PASEF (Bruker Daltonics), is now enabling users to combine HCP discovery and screening in one workflow, and accurately and reproducibly measure CCS values. Highly reproducible CCS values eliminate the need to include retention times (RT) in confidence measurements, enabling easy changing of LC methods, columns, flows, and other instrument parameters.

In instances that require deeper coverage of HCPs, samples can be analysed using a standard proteomics setup using nano ultra-high-performance liquid chromatography (UHPLC).

The Future of HCP Analysis

The low abundance of HCPs continues to present a challenge to the biopharmaceutical industry, and work on the removal of impurities has traditionally required specialised setups. PASEF scans implemented on advanced TIMS TOF MS instrumentation can be applied to HCP analysis using both routine analytical

and nanoflow configurations, to achieve sensitive detection with enhanced speed and data quality.

The depth of HCP identification provided by PASEF technology allows fingerprinting of biomanufacturing processes and ability to easily identify the effects of changes in these procedures. Sensitive, rapid, in-depth techniques for HCP analysis are driving the field of biotherapeutics, which is now transitioning into a new phase, thanks to decreased run times conferred by PASEF implemented on TIMS TOF MS. Such technologies are enabling biopharmaceutical manufacturers to accurately identify and quantify HCPs as CQAs and, therefore, comply with increasingly stringent regulatory guidelines.

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Freeze drying is helping meet the demand for probiotics and nutraceuticals across the food and health industries

A trend towards healthy eating has been growing globally for many years. Rising health concerns and the corresponding rising cost of healthcare itself has driven a move towards preventative healthcare and medicines. After all, isn't prevention always better than cure?

An increased consumer demand for foods with health benefits has therefore grown alongside increasing disposable incomes. Probiotics and nutraceuticals are not only meeting that demand, but consumers are beginning to see the health rewards from taking them. They are becoming more easily available, cheaper to produce, and consumers are becoming increasingly aware of their efficacy as clinical evidence builds. As a result, the global probiotics market is projected to reach $64.02 billion by 2022[1], and the global nutraceuticals market will reach $578.23 billion by 2025[2].

That's a LOT of people keen to maintain their health.

Where Do Probiotics and Nutraceuticals Come From?
Probiotics are living organisms such as bacteria and yeasts which are found naturally in many foods. When consumed in sufficient quantities, they can help maintain a healthy digestive system, lower cholesterol and even boost your immune system.

Nutraceuticals (also known as functional foods, medical foods, designer foods or nutritional supplements) are also naturally derived products, which provide additional health benefits aside from their basic nutritional value. Vitamins, minerals, herbs and enzymes can all have nutraceutical properties, and are believed to help combat such ailments as obesity, cancer, osteoporosis and cardiovascular disease.

The health benefits of foods containing these substances have been known for many years and aren't difficult to work into one's diet.

Probiotics occur naturally in fermented foods such as yoghurt, sauerkraut, tempeh, miso, pickles, olives and various cheeses. On the other hand, you can find nutraceutical vitamins, minerals and nutrients in many fruits, vegetables, beans, fish and seeds; all common supermarket staples. However, even foods that are rich sources of probiotics and nutraceuticals tend to contain relatively small quantities, especially when you're trying to achieve a specific health benefit.

With new technology we are now able to extract, concentrate, and consume probiotics and nutraceuticals in greater and more beneficial amounts, which would not be possible through consumption of the source food alone.

High Pressure Homogenisation (HPH) – which has long been used in the pasteurisation of milk – is one such technological advancement which is now being used in the production of nutraceuticals. Subjecting nutraceutical compounds to HPH

can help to maintain the bioactive compounds and can present a better alternative than thermal treatment for functional supplements containing heat-sensitive compounds.

Dynamic Vapour Sorption (DVS), a gravimetric technique that measures how quickly a sample absorbs a solvent vapour, is another new technology helping us to produce more stable nutraceuticals. It helps to determine upper and lower humidity limits for storage of products and reveals reversible and permanent changes in materials caused by increased humidity.

With these advancements, not only are we now able to produce nutraceuticals and probiotics in capsule form, we are also able to fortify other foods with them. Fortified or enriched foods include cereal, smoothies, nuts and seeds, milk... and even chocolate!

Production Challenges
Some of the difficulties in producing viable products include ensuring that they are stable, concentrated, and have an increased shelf life – all whilst maintaining their chemical structure to confirm they still provide the expected health benefits.

Manufacturers must also be able to yield large enough quantities of stable and viable product to batch produce as capsules, or to be added to dried foods as a fortifier.

The additional challenge with probiotics is that you are dealing with living organisms. It's vital that they reach the gut alive, or they will have no benefit at all.

The Solution – Freeze Drying

Freeze drying (lyophilisation) was initially developed for the preservation of medical supplies and has since been applied to the preservation of food – and now pharmaceutical and nutraceutical products.

Preservation of probiotics and nutraceuticals by freeze drying presents an effective solution to all of the above challenges. Not only does this method stabilise the product, but it concentrates large quantities down to a very small volume, increasing its potency and producing sizable batches. It also means the end product will not need refrigeration, making it very easy to transport and store.

This method also produces significantly more viable product than other processes, especially when it comes to probiotics. Many more bacteria survive the freeze drying process than with other preservation methods – but they must be protected against the additional stress that freeze drying puts on bacterial cells with a cryo-protectant such as trehalose, sucrose, glycerol or skimmed milk powder.

Whilst potentially cheaper and faster preservation methods exist, such as spray drying, vacuum oven drying and fluid bed drying, none can match freeze drying when it comes to maintaining viability of cells. With alternative methods, high temperatures can cause chemical and physical changes to the product, whereas lyophilsation preserves them with low water

activity post-lyophilisation; this results in no fundamental changes to the item's make-up. Freeze-drying is, therefore, the most commonly used form of probiotic dehydration.

How Biopharma Group Can Help

Freeze drying is our speciality at Biopharma Group. From equipment to product and process development, maintenance and validation to analytical equipment, we provide a complete package. If you are developing a new nutraceutical or probiotic product for the market, we could be your ideal partner.

We are the exclusive distributor for Cuddon Freeze Dry in Europe – a world-respected freeze drying specialist manufacturer with a high level of practical experience, developing an array of different models and configurations to satisfy a broad range of industrial purposes.

Cuddon freeze dryers allow you to shape a final product of superior quality, in a commercially viable machine. The capacity of the units, vacuum set-points, and variable shelf temperature options can be determined according to user needs.

Unlike some other freeze dryers on the market, geared towards the process of food or nutraceuticals, Cuddon's range provides accurate shelf temperature control at both positive and negative temperatures, which can lead to a greater product yield and efficacy overall.

In addition, with the largest Cuddon model offering ice condenser capacities up to 1500kg, operators can rest assured that should they ever seek to scale-up production throughput, Cuddon equipment can offer a solution.

Furthermore, a touch screen allows you to program and save recipes, record/analyse data, as well as operate/remotely monitor the lyophilisation cycle; these are all important aspects many operators demand in contemporary processing environments

The versatility of Cuddon freeze dryers makes them the perfect choice for probiotic and nutraceutical preservation.

If you're interested in exploring how a Cuddon freeze dryer could assist in your nutraceutical production, please get in contact today: **+44 (0)1962 841092 or via bps@biopharma.co.uk**

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Enhancing Cell Line Development and Streamlining Processes through Single-cell Technology

Innovative approaches that leverage single-cell analysis platforms, such as the Cyto-Mine® (developed by Sphere Fluidics), are removing significant barriers and boosting productivity within complex cell line development processes to transform delivery of novel biotherapeutic molecules. Such advances are enabling biopharmaceutical companies to swiftly transition from the initial transfection phase through to development of highly productive cell lines, delivering product concentrations of up to 10g/L, within timelines of approximately 10 weeks.

Leading companies in this area are increasingly seeking methodologies that optimise production of biological medicines via specialist contract development and manufacturing organisations (CDMOs), who offer technical expertise and capacity in cell line and process development. This enables biopharmaceutical companies to make more efficient and effective use of existing resources, while facilitating faster transit of therapeutic molecules into clinical development. Consequently, in this exceptionally competitive environment, CDMOs are under ever-increasing pressure to deliver robust and productive cell lines within shortened timeframes, while maintaining excellence concerning biologics product quality.

Antibody-derived Medicines and the Evolution of Biotherapeutics

The field of biological medicines is an exciting and rapidly changing area of drug development. Many existing biological drugs have already transformed the lives of patients living with life-limiting and debilitating diseases (e.g., cancers and autoimmune diseases). These complex and highly potent molecules have provided the drug development industry with multiple challenges, particularly concerning consistent production and expression capacity in cellular systems, as well as provision of quality assurance data for regulatory bodies.

Biological medicines have historically comprised monoclonal antibodies. However, emerging biotherapeutics have begun to focus on novel antibody fragments and bispecific antibodies, which, compared with standard monoclonal antibody molecules, are often more challenging to produce in stable cell line expression systems at high enough titres for commercial drug development programmes and large-scale manufacture. The growing urgency for new and unique therapeutic molecules has prompted biopharmaceutical companies and CDMOs to seek technological platforms that provide better predictive screening capabilities and higher throughputs, as current technologies do not deliver the level of efficiency required to meet demand.

Regulatory agencies, such as the Food and Drug Administration (FDA), require detailed evidence of monoclonality for cell lines producing biopharmaceutical agents. This means that companies must prove that the cell line producing the therapeutic molecule is derived from a single parent cell. Failure to meet these standards for monoclonality may result in enforcement of additional manufacturing controls, leading to costly delays to clinical trial programmes and market authorisation. Conventional screening techniques, such as colony picking and limiting dilution cloning, are time-consuming and restricted concerning the number of cells that can be processed within a given period. This represents a critical and rate-limiting step in the drug development process. Crucially, traditional approaches struggle to provide visualisation of individual cells upon initial seeding, which means that demonstrating monoclonality can be difficult and may not be possible in some cases. Higher throughput screening technologies, such as fluorescence-activated cell sorting (FACS), have become increasingly utilised over recent years in an effort to make cell line development processes more efficient. However, these systems can be harsh on delicate cultures as cellular suspensions are driven through a screening platform under pressure and current platforms are only able to measure antibodies that are bound to the cell membrane, rather than secreted proteins. They also require a great deal of technical expertise and training to use effectively in practice.

Optimising Mammalian Cell Line Expression Systems

Cell culture processes may be developed and adapted to yield high protein titres and tailored to enable expression of challenging molecules (e.g., bispecific antibodies and immunoglobulin fusion molecules). Timelines are critical in this highly pressurised and commercially driven field of the pharmaceutical industry. Cell line development processes must balance speed with delivery of high-quality biologic products and providers of these services should have the in-house capability to adapt workstreams to accommodate a wide range of molecule types.

Stable mammalian cell lines provide a well-accepted model system for recombinant protein production. Chinese hamster ovary (CHO) cells are among those host cell lines preferred for production of biological medicines [1,2]. They can produce fully humanised recombinant proteins displaying post-translational modifications that are compatible with those occurring in human cells (e.g., glycosylation)[1]. As a result, proteins produced in these cellular systems are likely to be active in humans[1]. CHO cells also offer flexibility as they can be cultured in suspension, serum-free chemically defined medium and scaled up for long-term manufacturing purposes[1,2].

A host cell line, taken from a fully-characterised and documented cell bank that has been produced in compliance with Current Good Manufacturing Processes (cGMP) and tested for the presence of adventitious agents, is advantageous in ensuring quality of the final product. During cell line development, transfection will result in a heterogenous cellular population and cell lines will be selected based on desirable characteristics/attributes.

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Choice of expression vector is a key aspect that can influence the success of recombinant protein expression in mammalian cell lines. Cell culture specialists must design an appropriate multi-component vector that optimises transcriptional regulation, via elements such as promoters or enhancers, and translation processes through elements such as initiation sequences, leader sequences and polyadenylation signals. Inclusion of an appropriate selectable marker gene (e.g., DHFR, a metabolic selection gene) is useful in identifying cells expressing the gene of interest and provides a critical detection flag that can be rapidly recognised during screening stages in cell line development.

Harnessing Single-cell Technology to Improve Efficiency and Ensure Quality

The application of emerging single-cell analysis technologies, evolved from automated microfluidic systems, has brought an exciting new dimension to cell line development and production of recombinant proteins. Single-cell platforms that harness picodroplet technology provide a means of implementing one-step cloning techniques in stable mammalian host cell lines, improving accuracy and efficiency of screening and culture development. Pools of transfected cells are isolated as single cells and encapsulated within individual picolitre-sized aqueous droplets in a biocompatible carrier oil (picodroplets). Each picodroplet provides a controlled, defined and adjustable environment that maintains cell viability (Figure 1)[3]. Single cells and their secreted proteins may be screened accurately and rapidly using this technology as the proteins are trapped within the picodroplets, making them easily accessible for analysis and characterisation.

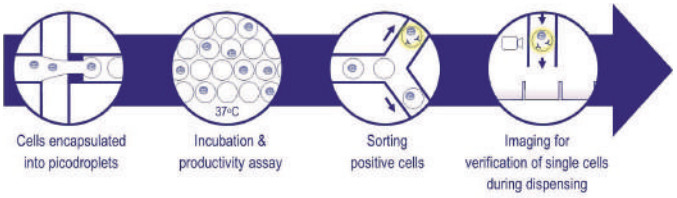


Figure 1: Integration of productivity assay screening, sorting, isolation and verification using a fully automated picodroplet microfluidic process

Single-step cloning processes remove bottlenecks in cell line development services, which are now only limited by cell doubling times. Historically, a two-step cloning process was implemented, which required manual picking of colonies, limiting dilution cloning and analysis using semi-solid agar colony screening. Fully automated single-cell platforms facilitate the single-cell isolation and productivity assessment of a greater number of cells in one simple step. The miniaturisation of assays and the integration of multiple steps in the cell line development workflow allows analysis that may have once taken weeks to complete to be undertaken within one day. Approximately 200,000 individual cells can be screened in a matter of hours using single-cell technologies, compared with around 10,000 cells with manual clone picking and analysis techniques. In addition, there is no need for cells to be plated during this process as they can be screened, sorted and verified while encapsulated within the picodroplets. Screening may also be conducted at an early stage so that only cells expressing the protein of interest are taken forward in the development process, providing major benefits concerning efficiency and use of resources. The new approach streamlines workflows; procedures that would previously have taken around 25 weeks to complete can be delivered in approximately 10 weeks (Figure 2). Protein expression titres of 10 g/L for monoclonal antibodies can be obtained using this methodology.

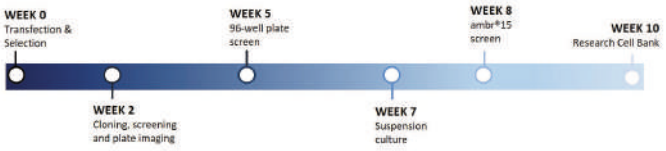


Figure 2: Example timeline highlighting the streamlining of workflows using mammalian cell line engineering technologies in combination with single-cell picodroplet innovations

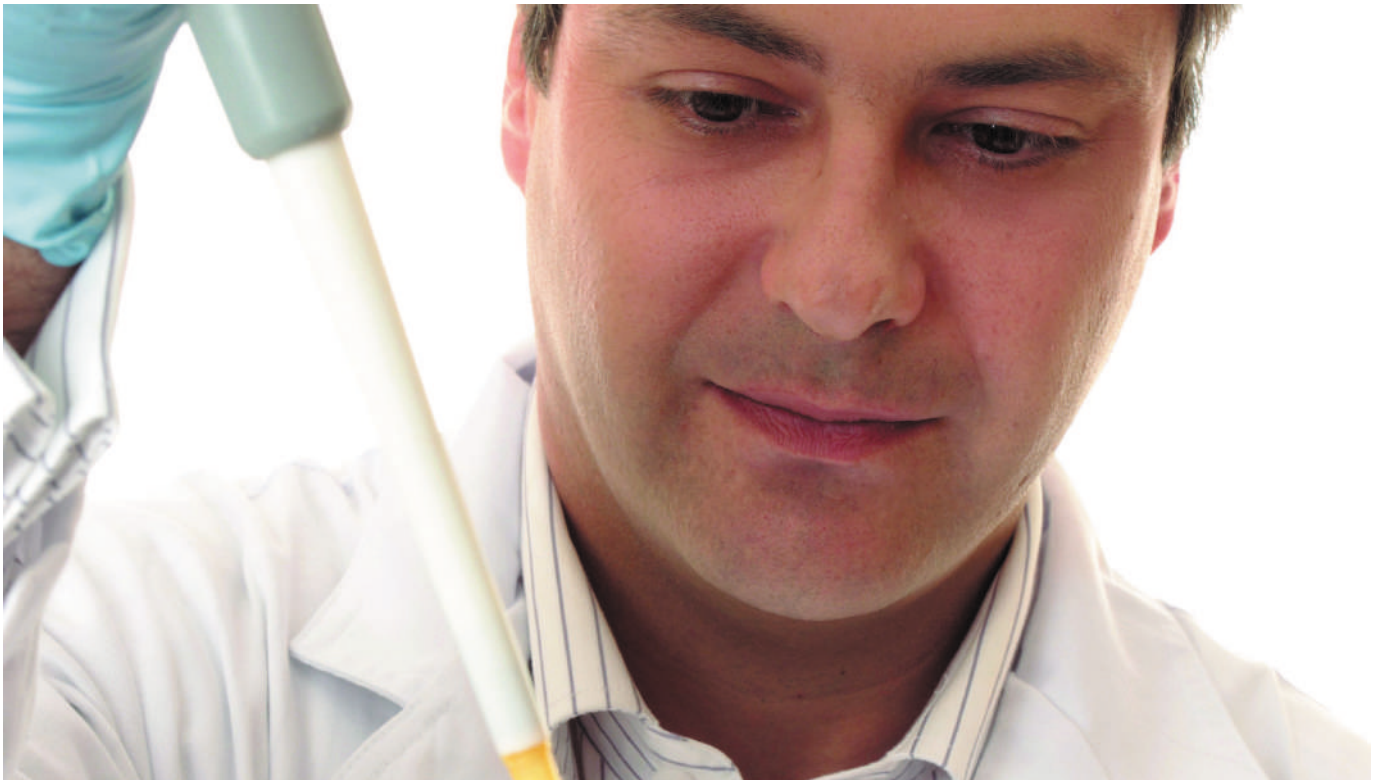
Analysis of single-cell productivity is possible using picodroplet technology, allowing the best performing cell to be selected and taken forward. This is particularly important when scientists are seeking those rare cells that produce large quantities of antibody, antibody fragments or other novel molecules that are typically very difficult to express in cell lines. Overall product quality and efficiency of production/manufacture are subsequently likely to be enhanced. The ability to visualise single cells also supports faster and more precise demonstration of monoclonality in line with regulatory requirements.

In addition to the overall benefits observed in terms of workflow efficiency and productivity from a CDMO or commercial perspective, the new approach provides greater freedom from laborious manual procedures for scientists working in the cell culture and process development environment. This allows them to apply their specialist skills and expertise to improve other important aspects of their work that may drive better service delivery. Scientific teams can undertake a greater number of projects during a given time period, while spending less time at the bench performing basic tasks. Unlike more traditional and complex screening platforms, automated single-cell technologies are designed with integrated user-friendly software, that requires relatively little specialist training to understand and implement effectively.

Fine-tuning Future Biotherapeutics

While picodroplet platforms clearly have a role in enhancing cell line development services, it is easy to predict further areas of potential for this technology in biotherapeutic development. Antibody discovery is a key area that would benefit from single-cell analysis systems, empowering scientists to swiftly screen vast molecular libraries to identify high-specificity antibodies that recognise novel cellular targets. Automated display and capture systems could allow interactions between secreted antibodies and multiple panels of specific antigens to be examined in high throughput. This would provide an excellent starting point for subsequent cell line development.

There is also some evidence that CHO cells grown in picodroplets demonstrate higher levels of antibody secretion, compared with those grown in bulk flask environments (Figure 3)[4]. In a rapidly expanding and competitive marketplace, biopharmaceutical companies are seeking to combine quality with capacity. A technology that enables greater and more efficient productivity, while supporting quality improvements, represents a very attractive proposition for CDMOs striving to meet the needs of biopharmaceutical clients as well as satisfying the requirements of regulatory bodies.



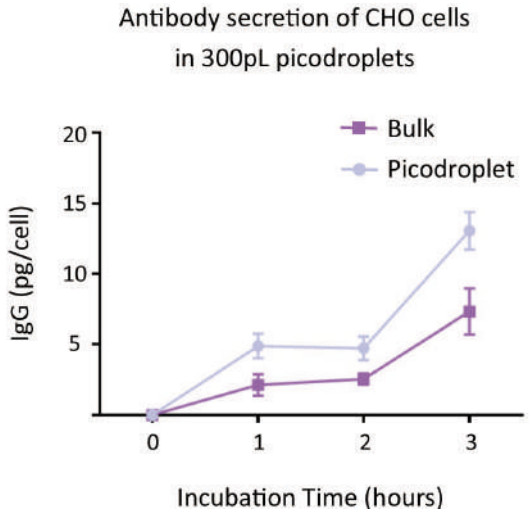


Figure 3: CHO cell antibody secretion in 300 pL picodroplets versus bulk production in a shake flask

The incorporation of single-cell technologies within specialist advanced mammalian expression systems has already begun to expand the possibilities for recombinant protein and biotherapeutic production. Looking to the future, the marriage of such technologies could revolutionise timelines for the development of ground-breaking new medicines and management of complex diseases.

The benefits of single-cell picodroplet technology in cell line development and biotherapeutic production

- Processes are streamlined and simplified (e.g., moving from a two-step cloning process to a single step)
- Monoclonality can be assured as the single parent cell can be visualised
- Higher numbers of cells can be accurately screened for productivity, compared with multi-step manual procedures (200,000 cells *versus* 10,000)
- Timelines are shortened, customers receive their cell line more rapidly, and new medicines may reach clinical development at greater speed
- Efficiency is increased and more projects can be delivered within a given timeframe
- Fully automated single-cell platforms enable scientists to focus on other important aspects of their work

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How to Gain the Greatest Blockchain Value for Your Business

To say that business is "undergoing significant change" in the 21st century does not do justice to the chaotic upheaval and breakneck speed that you as an enterprise executive must fight to keep abreast of. Global marketplaces, international supply chains, a web of partners, rigorous oversight, and increasing consumer demands all contribute to the complexity and challenges you face.

Yes, technology is coming to the rescue, embodied in automation, artificial intelligence, machine learning, biometrics, Blockchain, and the like. However, at first, these technologies tend to contribute to the confusion as businesses strive to understand, implement, and benefit from them. To bring much-needed clarity and direction, let's take a closer look at one of these leading-edge technologies: distributed ledger technology, commonly referred to as Blockchain.

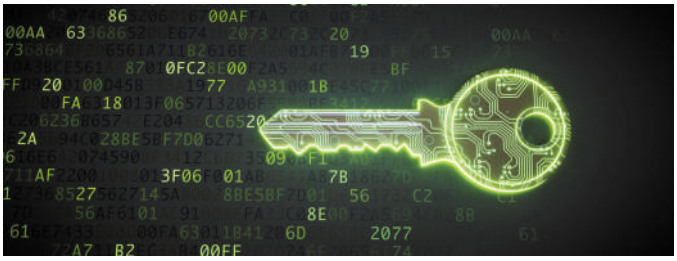
Understanding Blockchain's Benefits

Blockchain can be described as an incorruptible transaction ledger. Information stored in a Blockchain can be added to, but an attempt to change previously-stored data is immediately evident to all participants. The nature of a Blockchain delivers multiple benefits for businesses:

- Transparency. All transactions recorded in a Blockchain platform are recorded and accessible to all parties that need the information. Permissioned Blockchain ensure that commercially-sensitive information is visible on a need-to-know basis while enabling the participants at large to see data that is important to all.
- Data integrity. Since Blockchain is a decentralised system, data is recorded in multiple nodes that must reach consensus in order to commit the data to the chain. This ensures that the data is reliable and accessible.
- Information immutability. The complex nature of the cryptographic algorithms and the requirement for the nodes to come to consensus before adding information to the chain ensures that, once written to the Blockchain, the data remains unchanged. While it is theoretically possible for a bad actor to try to hack the Blockchain, the sheer amount of computing power that it would take to de-encrypt, modify, re-encrypt, and achieve consensus in the system makes this highly unlikely.
- Security via decentralisation. Since the information stored on the Blockchain platform lives on many servers, a single breach cannot impact the information stored on the Blockchain. Indeed, an actor would have to overtake 51% of all of the servers on the platform worldwide in order to change the information.
- Fewer intermediaries. Blockchain enables peer-to-peer transactions and can eliminate third-party "middlemen" in the process. This reduces the cost of doing business by allowing businesses to interact directly without paying a third party.
- Customer-centricity. Blockchain democratises trust in both business-to-business (B2B) and business-to-consumer (B2C) transactions. Business partners and customers have more power than ever now to demand changes. By working together in an environment that enables transparency, Blockchain provides business partners and customers with clarity into business activities and encourages a sense of trust.
- Automatic execution of business protocols. Smart Contracts enable members of the Blockchain community to agree upon the requirements for each business process. When the requirements are fulfilled, the Smart Contract immediately executes the terms – whether that be a transfer of payment or the release of goods.

For example, a Blockchain can help secure a pharmaceutical supply chain that consists of multiple vendors by facilitating the sharing of information, improving transparency and collaboration for all stakeholders. This can prevent therapeutics from being diverted into the black market. On rare occasions when there is a problem with the therapeutic itself (e.g., a contamination or a temperature breach that makes the drug ineffective) the information is immediately available and action can be taken. This protects public health and safety and enables manufacturers to change their plans to address potential shortfalls in availability. Regulators also have clarity into the issues that occur and can intervene in near real time to prevent the release of spoiled product and trace the source of any issues or diversions.

Blockchain has the potential to transform not only individual businesses, but also entire industries and society as a whole.



Consider sustainability initiatives worldwide. A company that has implemented Blockchain has a clear record of where and how the raw materials for their products are sourced. Activities that violate national and international law can immediately be identified, and the company can either take action to correct the vendor or cease working with the vendor. On a larger scale, once a violator is known, this information can be shared with the industry at large. For example, if a raw material vendor is violating the ethical and environmental standards for palm oil production, this information can be shared with other companies participating in the Blockchain, resulting in that vendor being excluded from conducting further business until they change their production methods. On a global scale, this information can be made public and increase pressure on raw material vendors to engage in ethical and legal practices, essentially enforcing the laws by excluding offenders from making a profit in the industry.

Incorporating Blockchain into Your Business

With such an array of benefits, it can be tempting to assume that Blockchain can and should be used for anything and everything. Strategising your use of Blockchain, however, is crucial. If you have processes that are performing well for you, there may be no need to replace them with Blockchain technology. Trust what is working.

Some of the areas where Blockchain has been demonstrated to deliver value include:

- Supply chain management, where multiple parties must interact seamlessly with one another, and where failure at one point can impact the product and the bottom line. For instance, the average medicine is transferred between nine or ten vendors from the time it leaves the manufacturer to when it reaches the pharmacy. Currently, the information on shipments is scattered between all of these actors, making it very difficult to track the source of an incident that either destroyed the drug's efficacy or enabled it to be sent to the black market. If all members of the pharmaceutical supply chain are sharing a Blockchain, then any deviations can be immediately identified and resolved without endangering the public at large.
- Financial transactions, in which establishing and maintaining trust among companies and/or consumers is critical. In the developing world, many people do not have access to a bank and therefore have no credit history. This means that, even though they may have the initial money needed to start a business, they cannot receive a loan because there is no record of their reliability in the financial system. Blockchain can be used to enable people who would otherwise not have access to a bank to open accounts, create a credit history, and receive the loans they need. This enables economic growth on a large scale in areas that need it most.
- Executing contracts in near real time, as companies can use Smart Contracts to collaboratively agree on the terms for a contract and inculcate them with executable code that can automatically run on the Blockchain. Smart Contracts insert this business logic into the Blockchain so that payment can be triggered immediately upon the receipt or execution of terms in a contract. Smart Contracts will also prevent payment from being released if the contract terms are not met.
- Compliance with national and global regulations, as the combination of immutable records on the Blockchain and business logic executed through verifiable Smart Contracts will ensure that business is being conducted in alignment with legal standards. This ensures that companies will not face fines for violating the law and enables transactions to be conducted more quickly and with greater transparency.

It is best to start small. Identify a project that will generate a return on investment (ROI) in the shortest amount of time. An eight-to-twelve-week engagement that proves the ability of Blockchain to solve a key tactical or strategic issue is appropriate for a proof of concept (POC). This allows you to become comfortable and familiar with the technology and delivers a "win" for your business without committing too much in the way of time and resources. A successful POC can be expanded into the pilot phase, in which the system is iteratively improved upon to ensure that it continues to deliver value.

Another key consideration when deciding whether or not to implement a Blockchain platform is the long-term ramifications that this may have on the relationships that IT must create in order to be successful. As an example, the implementation of Smart Contracts may require the advice of the legal team to ensure that the technology meets federal and international law. Also, moving from a centralised to a decentralised system will bring with it the need to rewrite governing processes that could impact workflow and supporting infrastructure.

Blockchain is poised to disrupt the pharmaceutical supply chain as we know it. The hurdles can and will be overcome, though it will take the consolidated effort and cooperation of stakeholders across the supply chain – not just of IT experts tackling the complex programming involved.

Camille Diges

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Connected Labelling: The Holy Grail that Continues to Elude Life Sciences?

If there is one content management challenge that is causing compliance headaches for international life sciences organisations, it is labelling. Over-reliance on manual processes to cross-check content inter-dependencies, and to manage requirements for each country whenever something changes, leaves companies at risk of delays to market – or even registrations being withdrawn. AMPLEXOR's Agnes Cwienczek considers how they can turn this situation around.

As life sciences companies' R&D ambitions grow, as they expand into more markets, and as regulators continue to strengthen safety requirements to protect patients from harm, the global labelling management burden has soared. Each time that the benefit/risk profile or safety factors change, or as regional or local regulatory agencies update their standards, international biopharmaceutical firms must react swiftly with accurate, compliant labelling for all affected markets. If they do not, they risk delays to shipping or even to keeping their product registrations on the market, with the obvious impact on sales, revenues and public confidence.

Balancing market opportunity, risk and cost is becoming increasingly tough to do, so it is unsurprising that organisations are keen to bring the situation under control. Given that all of these market conditions are set to intensify rather than diminish, finding a solution sooner rather than later has become a priority. Managing labelling across multiple markets internationally is challenging enough, but as the frequency of changes increases – due to ever-growing product complexity, heightened pharmacovigilance, and new regulatory specifications – the demands soar exponentially.

There are a number of practical reasons for the rising labelling management complexity, and much of this has to do with the way content interdependencies are managed. That is, how a change anywhere along the safety-regulatory-manufacturing-supply chain continuum will affect all label content – from the organisation's global 'core data sheet', to patient-facing product information at a reference-country and dependent-country level.

The traditional way of managing all of this has been via a combination of legacy, purpose-specific systems and manual processes. Typically, tracking of label status and changes has happened in Excel spreadsheets or home-grown systems, and content has been updated on a country-by-country basis using dedicated, often standalone labelling tools. Even where such applications include some level of monitoring facility, the value of this has been limited by the fact that labelling tools are rarely – if ever – connected to companies' registration and submission planning and management tools.

As a result, central labelling teams have not found it easy to build up a clear, accurate and comprehensive picture of all labelling activities. Nor are they able to manage these systematically, end to end, irrespective of where the trigger for a change to the content might come from. In other words, it is difficult for them to efficiently map country/label interdependencies so that changes can be rolled out promptly and reliably wherever the new requirements apply.

The Dangers of Inertia where Change and Variance are the Norm

The risk of persisting with existing approaches is non-compliance, and the practical implications of this. These include delays to market and the very real possibility that product registrations could fail or be reversed. If the requirement is an urgent safety change – that a pack must carry a new warning, or a new contraindication must be included in the leaflet – companies must respond with effective action within 30 days, for instance. The timescale for safety-related changes is often strictly regulated, leaving little room for negotiations in the event that companies lack the 360-degree visibility across their global operations, or effective controls, which render them unable to perform a rapid and comprehensive impact assessment.

The ripple effects of changes aren't consistent, either, which further adds to labelling complexity. Latin American countries, for example, may be dependent on Europe for labelling convention for tablets, but on US product information for medicinal solutions. These jurisdictional variances in determining which guidance to follow, and precisely what to include, add to the management burden.

In addition to the risk of non-compliance, all of this adds up to a laborious and cost-laden workload for life sciences organisations, as they try to keep track of the latest implemented labels in all of their markets, and roll out each new set of changes, as applicable, within the required timeframes. Where submissions are bundled and/or split at country level in order to comply with local regulations or company internal needs and strategies, there are further considerations as companies try to maintain traceability – especially as changes trickle down to dependent countries, which may operate at arms' length to the main business.

Triggers for labelling changes can come at different points in the drug ecosystem, too. While a change to the benefit/risk profile of a drug will drive revised labelling requirements from the global core of the organisation, regulatory changes local to a reference country can drive a need for amendments both up and down the chain – up to the core, and down to dependent markets. So, any solution cannot be one-directional in its treatment of cascading changes.

Portals Only Solve Part of the Problem

Biopharmaceutical companies have tried to address some of the complexity and the expanding workload by creating

data-exchange portals between global and local functions – through which the different points in the international labelling management chain can collaborate on, and report back about, required changes and related progress. But even this relies too heavily on manual updates and process repetition.

Additionally, none of this takes into account the broader biopharma ecosystem: the safety-regulatory-manufacturing-supply chain continuum. Full labelling traceability, for the purposes of compliance, reporting, and safety/risk management, depends on content and processes being open to easy scrutiny right from one end of the chain to the other – from a safety signal, to the regulatory submission and approval, through to product receipt by pharmacies or hospitals. True end-to-end label and product tracking, and change management, ideally needs to encompass artwork updates, and follow-through into and beyond warehouses. In the event of a safety issue or compliance checks, the onus is on the drugs company to be able to demonstrate compliance, or to act swiftly to address batches of affected products which do not conform to the new labelling standard or contain up-to-date information and patient advice.

To date, this broader perspective and ambition remains largely aspirational. But achieving complete end-to-end traceability and control is something life sciences organisations are increasingly attuned to in their vision for process improvements.

Certainly, there is a growing urgency among companies to transform their labelling management activities. The bolder and more innovative firms become in their quest to expand their markets and portfolios, and the more they become subject to intensifying regulatory/public safety criteria, the more meticulous and efficient they must be in their monitoring and process controls.

The Future is Digital

A further factor in all of this is that, the more regulatory agencies increase their taste and flair for electronic records and use of digital systems for receiving, recording and reviewing product data and associated safety information, the more ambitious they are likely to become in their expectations and use of that data. This is especially the case as populations grow older, and disease profiles become more diverse, making the benefit/risk ratio harder to calculate. The likelihood is that traceability will become increasingly important over time, and that agencies will call upon the industry to cooperate by supply more structured and detailed data.

To get ahead of these trends, companies must update their approach to and means of managing labelling across a product's lifecycle. And indeed, many are responding now, with 3–5-year change programmes designed to create more joined-up, transparent and collaborative label management environments – which transcend country, departmental and even organisational boundaries.

What's most encouraging about such plans is that they allow for the bigger picture. They pave the way for a definitive source of labelling truth, in the form of central master data - from which everything else flows. They also embrace intelligent workflows – as an efficient and reliable means to assess the impact of label changes/calculate interdependencies; to drive through changes

with reduced manual intervention; and to facilitate greater collaboration across departmental, country and supply-chain boundaries.

The best-laid plans are cross-functional. They assume and allow for connection with global ERP/manufacturing systems; inclusion of local stakeholders in any tools and processes; and future automation – for instance, structured authoring of labels and patient information built from approved master content assets. The investment programmes are holistic in approach. They look at everything from how country-specific requirements are captured, the knock-on effect of changes across the global organisation, the interplay of different operational functions, and the potential for content re-use once 'fragments' or blocks of text/icons/images have been approved and filed in the master repository.

That companies are planning for this properly is very promising and will stand them in good stead to keep pace with developments as the pace of industry transformation continues to accelerate. These are early days, but there is much to gain by being thorough. First steps include determining where all the sources of data are and how best to connect them. With the right building blocks in place, firms will be better placed to realise their intentions. Moreover, they will finally be able to free themselves from the stultifying challenge that global labelling management has become.

Agnes Cwienczek

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The FDA is Shifting Focus to Both Ends of the Product Development Spectrum

The Food and Drug Administration (FDA) recently announced measures it plans to take in order to process and approve progressively more complex novel and generic therapies. There are some key areas expected to boom over the next few years, including two in particular. The first is novel cell and gene therapies, more specifically CAR-T therapy, a transformative new category of medicine. This new and complex area of development brings possible treatments for those diseases which, until now, have had a poor prognosis in terms of patient outcomes and offers pharma companies a new avenue of development. The second boom area, at the other end of the development spectrum, is generic medications. These will provide healthcare professionals with the option of less expensive solutions to diseases that previously may have been incredibly expensive to treat, helping to tackle the high cost of treatments to government providers, insurance companies and patients across the US. From the most complex innovations to the generic, the rapidly evolving regulatory processes mean that pharma companies have to work intensely to master the updated paradigms in order to maximise their FDA interactions and ensure fast product approval.

Technology and Capability Advances are Driving the Surge in Cell and Gene Therapy Submissions

Cell and gene therapy research and development has grown rapidly in past years and continues to accelerate. This is due to two key aspects: firstly, advanced technology, both biological and digital, has been made increasingly available to a wide variety of organisations from universities to pharma companies. This has allowed for greater understanding and visualisation of some of the intricate mechanisms behind disease that have previously been hidden. Secondly, an increase in investments from private and public sectors has provided pharmaceutical companies with the capability, scale and space required to investigate and produce hard to develop medicines. Additionally, the Center for Biologics Evaluation and Research (CBER), the regulatory body for cell and gene therapies within the FDA, will be providing a wide range of support for the development of these treatments. This support will include proactive scientific and regulatory advice for both researchers and manufacturers in the area of novel product development, as well as new guidelines on the development and approval of medicines within this field. The aim is that this additional support will ultimately allow new product submissions to be reviewed and approved more efficiently than is currently possible.

There has already been a surge of regulatory submissions in this area; the FDA had over 800 investigational new drug (IND) applications for cell and gene therapies on its books at the start of 2019 and it is expecting this number to rapidly increase, with a further 200 IND applications each year for the next ten years. By 2025, CBER are expecting to approve between 10 and 20 cell and gene therapies annually, a huge increase when considering that only around 20 therapies have been approved by the FDA to date. To help accommodate the inevitable increased pressure this will bring on the review and approvals process, the FDA is planning a significant expansion in personnel to oversee the clinical development process and review of cell and gene therapy applications.[1] There are also new, comprehensive guidance documents issued and planned to help pharma companies navigate through product development challenges. The FDA are specifically creating new guidance on innovative trial design and common manufacturing protocols, with the intention that these may then allow smaller groups, e.g. academics, to conduct their own clinical trials and therefore help develop even more novel cell and gene therapies. It remains to be seen whether these new trial designs will be embraced, however, but the pragmatism and sense of urgency by the FDA should be applauded.

One research area in particular receiving intense focus is chimeric antigen receptor (CAR-T) products specifically, which the FDA will need to nurture and support the most due to the huge medical impact that they are expected to have. This groundbreaking method of treatment reprogrammes the body's own T-cells so that they attack specific cells, e.g. cancer cells, directly. These treatments could potentially provide a cure for some of the hardest to treat cancers, such as B cell acute lymphoblastic leukaemia in children. However, the engineering and development of CAR-T therapies has proved by no means to be a straightforward process. The FDA has demonstrated its commitment to this promising area of medicine by developing a policy to clarify how it will apply its existing expedited development pathways, including breakthrough designation products, to products in the CAR-T cell and gene therapy classes. The FDA hopes to ease the development and review process by guiding companies in crafting their study design, data collection and regulatory submissions for these life-changing treatments.

Once a treatment has been through an expedited development process and is in market, the FDA would likely require post-market, confirmatory studies that provide real-world evidence and allow for larger data sets to help confirm the clinical benefit. These studies would provide additional evidence on the product's safety, efficacy or optimal use, and may be essential to further evaluate the risk/benefit of the product. It's important to remember that, no matter how impressive these treatments may first appear, there are nonetheless always some key safety concerns which must be considered in the post-marketing setting. Some CAR-T therapies have shown a risk of severe toxicity from cytokine release syndrome (CRS) in patients, so the FDA is conscious of being accused of pushing these therapies through their review too quickly or not requiring adequate post-marketing followup. To help manage this side-effect in practice, the FDA has included specific requirements that any hospitals or healthcare professionals prescribing CAR-T therapies must have received special training in order to be able to recognise, diagnose and treat CRS.

Easing the Development Uncertainties of Generic Therapies will Encourage Greater Competition

Generic medicines, at the other end of the economic spectrum to cell and gene therapies, are another key focus for the FDA. With appropriate competition, unbranded drugs can be marketed usually at a fraction of the cost of proprietary medicines, making them incredibly attractive to healthcare payers and patients. This is especially poignant when considering the current cost burden for prescription drugs for patients in the USA, who have the highest costs in the world, is around $1200 out of pocket for the average person per year.

The FDA is taking on this issue of drug affordability by further reviewing its priorities and putting a greater emphasis on generic drug applications.[2] The effects of this effort are expected to build on the already impressive increase in generic approvals, which reached a record number in 2018 with more than 1000 new generic drugs approved or tentatively approved. Efforts are also being made to ensure that the Orange Book, the compendium of proprietary drugs and their generic counterparts, provides the greatest benefit to patients, healthcare providers and generic drug developers: the FDA is currently making updates that aim to make it easier for all parties to understand which products have a generic alternative available in market, and conversely where generic competition is currently lacking. It is hoped that this will foster prioritisation of certain generic applications and help to expedite their availability.

In addition, the FDA has made significant progress in helping to strengthen and streamline the generic drug review process, providing scientific clarity and guidance to support the development of generics, including hard-to-develop complex products. There is also a push for shaming and calling out of any potential abuses of the system, where branded drug companies use tactics to delay market entry of generic competitors. By promoting generic competition, the FDA aims to help bring down the high prices of medicines in the US and help patients obtain and afford the treatments they need.

Changes Have Wide-reaching Implications for Trial Design and Duration in These Areas

The FDA's emphasis on these therapy areas will have a far-reaching impact within the pharmaceutical industry but in some cases the impact may not be felt fully for some time. Cell and gene therapies are paving the way towards treating some extremely rare diseases and conditions for which there are no treatments or where current therapies are lacking or suboptimal, but this in itself brings challenges. When conducting a clinical study for such rare diseases, the available patient pool is obviously much smaller. The FDA may in many instances allow an accelerated development pathway to be used for cell and gene therapies. This could allow drugs to be approved much faster as the submissions can be based on a surrogate or intermediate clinical endpoint that acts as a predictor of the real clinical benefit. For example, there will be new FDA guidance developed specifically for gene therapy products treating inherited blood disorders and neurodegenerative disease, conditions for which current treatments are particularly lacking. For CAR-T therapies specifically, the agency plans to recommend how manufacturing changes can be introduced during ongoing studies without requiring new clinical investigations and bridging studies.

This is Just the Beginning of the Evolution for the FDA

The FDA recognises that cell and gene therapy is an investigational area that is rapidly growing, and it will therefore require additional staff to handle the significant increase in applications for these important new medicines. This has led to a major hiring initiative, with the FDA planning to increase the number of dedicated clinical review staff by around 50 people, who will have the significant responsibility of reviewing these complex cell and gene therapies.

The FDA has taken significant steps toward removing potential barriers that could block the development and approval of generic medicines, and new guidance documents are being published on an almost monthly basis. The FDA continues to urge the pharma industry to meet with them to discuss development plans early on. New guidelines providing detail around the use of new analytic tools and tests that help prove the sameness of more complex generic drugs are currently planned for release. The FDA hope that increased communication will encourage companies to invest in the necessary science to prove comparability to branded drugs that would otherwise be too risky or difficult to replicate.

There are also plans for a new pathway for competitive generic therapies (CGTs), which would apply to drugs with only one approved generic in the Orange Book. Any application submitted as a CGT could receive expedited review by the FDA. Since real price competition only occurs after several generics are approved, the aim of CGT is to incentivise companies to develop and market new high-quality generic medicines to help bring down the cost of treatment and improve patient access.

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Andrew S. Verderame

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Innovations in Cold Chain Technology

Patient safety will always be the top priority of every clinical trial. Therefore, it is vital that the investigational medicine dispensed to the patient is pure, safe and effective, no matter what challenges are presented by packaging, storage and distribution requirements.

Many medicines have a stringent stability profile, where the acceptable temperature range in which the drug must be stored and distributed is strictly dictated to ensure the product remains safe and effective. This is becoming the norm rather than the exception, with large molecules and biologic medicines now playing a more prominent role in clinical development. Therefore, ensuring the biologics are optimally safe and effective is often an issue of sustaining the product at very specific temperatures, and maintaining these requisites despite what can be differing, and even extreme, environmental conditions.

In order to control the temperature conditions of the drug at every step in the supply chain, it is critical that trial sponsors consider a range of factors.

From the point in time that the drug is first manufactured through to end delivery to the patient, it will be exposed to a multitude of environments during its lifecycle. Stages of this will include initial packaging; storage at the distribution facility; transportation to and storage at the clinical site; and distribution to the patient.

At the conclusion of a clinical trial, returning and reconciling investigational medicinal product (IMP) is a further critical stage of the study and can in turn require similarly complex reverse logistics to ensure returns are properly processed. Trial outcomes can be impacted if returns are not processed efficiently and precisely, with full traceability and accountability of the returned product.

Monitors and Real-time Analysis

During the investigational study, traditional USB temperature monitors provide sponsors with the capability to identify any temperature excursions that may have occurred during shipment. These monitors have now advanced to offer tracking of additional internal conditions such as light and humidity, as well as shock and vibration. The tracker can then send the information directly to cloud storage once received at site.

Furthermore, GPS-enabled devices can tell you where your material is in transit and provide live updates. Although more sophisticated and therefore correspondingly expensive, real-time data collection monitors are readily available on the market and are certainly used in special circumstances. However, the real question is: what strategies can we use when we see that an excursion might be likely to happen? In many instances, there is not enough benefit to justify the expense of real-time monitoring, considering there still may not be enough time or resource saved by their use to mitigate the excursion before it happens – the product has already been shipped and cannot be easily and cost-effectively retrieved from the supply chain.

The technology of real-time temperature monitoring, while impressive, will not prevent an excursion from happening but can be of benefit if there are protocols in place to mitigate the consequences of an excursion. Once a problem has been detected, the options are limited depending on where in the route the shipment is located. The considerations would be to either send out a fresh shipment to replace the suspect product or possibly to re-route the shipment back to the origin. Even so, the latter option may not be enough to save the shipment contents – often invaluable and highly expensive drug product, due to its limited availability.

Shipper Qualifications and Innovations

For more sustainable temperatures within each shipment, a higher standard for shipper qualifications can be enabled, testing

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shipper performance against extreme temperature conditions that are much more stringent than the industry standard requirements. Once a shipment leaves the origin, the shipper is bound to face an array of environmental conditions depending on the weather and various global geographies.

With such variables in mind, several profiles can be developed based on the season. For example, a winter profile may account for extreme low temperatures on the tarmac in, for example, the UK or Germany, and yet above average hot temperatures at a destination location that may be nearer the equator, such as in the Ukraine or Saudi Arabia.

A second profile for summer would account for warmer temperatures overall but might also include considerations for a short period of mild environmental temperature when in flight, and extremely high temperatures throughout ground transit.

The shipper is chosen daily based on the current weather forecast at each point in the supply chain. In turn, each parcel in the consignment has a packaging configuration and cooling method to keep the drug in temperature, whether it be a water-based gel, phase change or dry ice. The chosen configuration will mitigate any risk the environment may pose on the investigational product inside.

As well as temperature, these shipper configurations will also take local infrastructure demands into account. Clinical studies are expanding now across the world, to places such as Northern Africa and the Middle East, where trials in the past were typically not conducted. These locations add a further level of consideration when choosing a shipper configuration; they might have less developed transport networks so ensuring safe and speedy shipments from central locations to perhaps more remote clinical centres or doctors' practices might present a challenge. For these reasons, considerations need to be made to allow for extra time in transit in warm temperatures. In these situations, it is important to use shipping systems with longer prequalification times, or even specific shippers that have been qualified to exact payloads – as opposed to using a standard prequalified shipper.

Another solution would be to work with couriers which can provide specialist temperature-controlled vehicles to enable suitable transportation of medicines to more remote areas. In these situations, this removes sole reliance on the shipper to maintain temperature but can add the extra assurance of an active system in the refrigerated vehicle that keeps the temperature precisely where it needs to be during its journey.

One long-awaited shipper option has been developed to address concerns over waste materials – helping to answer an increasing need for better environmental practice for those operating in the pharmaceutical sector. Shipper configurations now include a reusable option, providing a greener alternative to traditional shippers. Traditional insulation and cooling materials use a significant amount of packaging space and weight and leave the receiving site with the responsibility of disposing of all waste materials – which often poses a challenge. With a reusable option, the site is able to send the shipping components back with the carrier who then hauls away the materials and, after a thorough cleaning and inspection, either recycles or reuses the materials.

The results of implementing an option to reuse materials is often cost-neutral, as the materials are lighter. These shippers utilise vacuum insulated panels (VIP) as the means of maintaining temperature and are specifically designed for efficient and compliant refurbishment. Although the extra cost of moving the empty shippers back to the courier's central location increases the price, the reusable aspect and the lightweight material often offsets the cost of the additional trip. This principle holds especially true when trading shipments over long distances. For example, trading shipments between locations in the US and Australia can be especially cost-saving and reduce pallet-loads of waste.

Limitations of Kitting

When considering clinical trials held in geographies with limited storage facilities (which can be a common occurrence in Europe where hospitals in urban areas tend to be older and have tight quarters) it is important to consider the footprint of your package design. The growing popularity of kitting, for example, may cause the investigator difficulty once on site if storage facilities, especially refrigerated storage space, are scarce. The kit may add additional, unnecessary bulk for items that do not require temperature control or monitoring. The ideal direction is to care for the cold chain product in a specific temperature-controlled environment and handle the ancillary items separately to optimally utilise valuable and limited cold storage. For example, adding syringes and needles to a kit will look nice and may provide some convenience, but kitting them together is not recommended where storage facilities are in high demand. This will also save the sponsor the cost of cold chain storage and distribution for items that do not require those conditions.

Clinical trial supplies pass through multiple checkpoints before reaching the patient, and careful planning using traditional and non-traditional methods to mitigate risk is key to ensure the patient receives treatment that is safe, pure and effective for the best possible outcomes. With the discovery of new medicines, new challenges in the supply chain often arise. Close and trusted partnerships between client and vendor ensure solutions can be developed to surpass each challenge. The industry is always changing and fortunately, better solutions are being introduced to effectively handle these challenging products.

Brian Keesee

Brian Keesee, Vice President & General Manager of PCI's Global Clinical Operations & Supply, has spent his entire career in the pharmaceutical industry. In his current role, Brian is responsible for global clinical operations and project management, including PCI's state-of-the-art packaging and distribution facilities located in Rockford, Illinois and Bridgend, Wales. Prior to his role at PCI, he held leadership positions in compliance, operations management, and project management within the pharma industry. Brian has managed large global clinical studies and offers extensive experience and expertise in supply chain management, logistics, clinical package and label design, project management, cold chain technologies and IRT. Brian brings a unique perspective based on his global project management knowledge from his prior positions within the clinical supplies industry, his compliance and operations roles at AmerisourceBergen Drug Corporation, and his involvement in a Lean Six Sigma Black Belt Programme.

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