APRIL 2023 Volume 9, Issue 3

CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON:
Vector bioprocessing & materials

Guest Editor: Andrew Tustian, Regeneron Pharmaceuticals

CELL & GENE THERAPY INSIGHTS

Volume 9, Issue 3

CONTENTS

SPOTLIGHT: VECTOR BIOPROCESSING & MATERIALS
ANALYTICS CHANNEL EDITION
LATEST ARTICLES

Spotlight

Vector bioprocessing & materials

Analytics Channel

Latest articles

CELL & GENE THERAPY INSIGHTS

VECTOR BIOPROCESSING & RAW MATERIALS

SPOTLIGHT

INTERVIEW

Exploring the evolution of upstream & downstream viral vector processing

David McCall, Commissioning Editor, *Cell & Gene Therapy Insights,* speaks to **Francesca Vitelli,** VP, Cell Therapy and AAV Process Development, Intellia Therapeutics

FRANCESCA VITELLI brings over 20 years' experience to her current role leading viral vector and cell therapy process development, manufacturing sciences and vector core services at Intellia Therapeutics. She has held technical leadership roles in Process and Analytical Development, Manufacturing, and Tech Ops at a multinational CDMO and regenerative medicine start-ups and has worked with cell types including HSCs, iPSCs and T cells, viral vectors including AAV and LVV and many supporting production systems. Prior, she was an assistant professor of molecular development and disease after her PhD in medical genetics. Designing and deploying innovation to improve the lives of those impacted by genetic disease has motivated Francesca's journey through academia, entrepreneurship and industry.

Cell & Gene Therapy Insights 2023; 9(3), 287–294

DOI: 10.18609/cgti.2023.045

Q What are you working on right now?

FV: I joined Intellia Therapeutics to head up the viral vector and cell therapy process development and manufacturing sciences team, supporting both our *ex vivo* efforts and our *in vivo* insertion and internal vector core needs. Currently, the approach at Intellia is to outsource most manufacturing to our Contract Development and Manufacturing Organization (CDMO) partners, though we have taken steps to secure in-house production for some of our components.

Intellia is a full-spectrum genome editing company with a toolbox of both editing and delivery solutions. Our aspiration is to unlock the full potential of CRISPR editing by taking both *in vivo* and *ex vivo* approaches. The *in vivo* approach is two pronged. On the one hand, we seek to reduce expression of harmful proteins through gene knockout. In that area, our two current lead candidates in the clinic, NTLA-2001 and -2002, have shown exciting initial clinical data. We also have a wholly owned gene augmentation approach through our insertion programs - NTLA-3001 for alpha-1 antitrypsin deficiency, and another in collaboration with our partner Regeneron for hemophilia A and B. Our insertion programs use adeno associated virus (AAV) to deliver promoter less DNA templates encoding the therapeutic protein to the liver. The CRISPR tools are also delivered to the liver by lipid nanoparticles (LNPs) where they generate a double-strand break in the albumin locus. Using the cells' intrinsic repair machinery, the template is inserted into the site of the cut. Through the endogenous regulation of albumin expression, we achieve controlled levels of expression of the functional protein in an accurate and precise manner.

We also use both an AAV vector and CRISPR in our *ex vivo* programs to engineer T cells. In this approach, we use a combination of lipid nanoparticles (LNPs) and AAV6 to engineer a differentiated and powerful allogeneic editing approach. T cell transduction in order to express the chimeric antigen receptor (CAR) or the T cell receptor (TCR) is achieved through AAV6 template insertion. CRISPR demonstrates a double-strand break in the TRAC locus and using homology-directed repair, we achieve precise, accurate, and stable transgene insertion. Although Intellia is not known as an AAV company, AAV development, analytics, and manufacturing are important components to our success. The AAV process development and manufacturing sciences team focuses on developing commercially-minded, robust processes that can perform reliably to yield quality product every time we manufacture.

Q Your most recent two roles have seen you head viral vector process development at a major CDMO (Lonza) and now a major gene therapy biotech in Intellia – what would you say are the key trends in how each sector is evolving, and what might this mean for in-house vs outsourced manufacturing strategies in the viral vector field moving forward?

DOI: 10.18609/cgti.2023.045

FV: Several years ago, we saw a huge crunch on the capacity side, but now we are seeing a great abundance of offerings after CDMOs have invested heavily in capacity and viral vector innovation. The question of build-versus-buy-versus-blend is not new. The value proposition of internalized manufacturing could change for some companies as an asset progresses through its clinical stages, either to provide more control or to diversify supply. New offerings of clean rooms for hire where developers provide staff are interesting solutions for early clinical stages.

With this boom in offerings, there are now more solutions available than ever before. As a developer, this is great because we can pick between companies with commercial experience, which is useful if you have a later-stage asset. Other vendors are differentiating in terms of their offerings providing the potential for a one-stop shop to provide plasmids, mRNA, viral vector, and the *ex vivo* cell therapies. Still others are heavily focused on a full in-house suite of analytical offerings, built out alongside their manufacturing platforms, or trying to incorporate tools like process analytical technologies (PAT) or machine learning and artificial intelligence even from early stages to facilitate operational excellence. This is in the spirit of Quality by Design (QbD), which is now coming within reach of the cell and gene therapy industry given our deeper product characterization and understanding.

Interestingly, due to this abundance of supply, we see some CDMO differentiating themselves – for example, focusing on suspension transient transfection and demonstrating scale-up at 2000 L on their own dime and claiming the ability to scale to 5000 L in single-use systems. This is important because it provides a demonstrated and speedy path to good manufacturing practice (GMP). Others are willing to take on anchor clients, or to adopt new models such as risk-sharing approaches in exchange for equity or other considerations, both of which could be appealing to new biotech companies.

Despite this change in the offerings landscape, one thing has not changed – the importance for CDMOs to be engaged and truly partner with developers with trust and transparency from the outset. As the field matures, there will be little room for inflexibility, poor communication, or poor execution. The rising partnership bar may shift the bottleneck from previous capacity issues to current capability issues, where experience, expertise, and willingness to be flexible are key. However, this may also exacerbate the problem of the lack of a skilled workforce. We could be at a tipping point for greater sustainability on the personnel training side.

Q What are the pros and cons of the growing menu of adherent and suspension-based bioreactor technologies, and what are the key related issues and questions for the industry?

FV: The key goal for the industry is to reliably manufacture scalable, cost effective, and high quality product. A key challenge for the industry is the lack of, and

"...several tool providers and CDMOs are working on rep/cap plasmid design and helper plasmids to boost productivity and reduce presence of process- and product-related impurities which are exciting ways to control costs by increasing yields and productivity and quality."

unlikeliness to reach, harmonization on a singular approach to this goal, making consolidation of efforts to unravel the issues somewhat complex. Today, developers are using a number of solutions each with trade-offs: transient transfection with two or three plasmids in adherent or suspension HEK293 cells, insect cell-based systems, and HeLa-based producer cell lines are the most common. Factors that play into the decisions include speed and ease of producing material for first clinical studies, expertise in-house or at a CDMO partner, overall projected viral genome demand and process scalability, expected tropism of the product and any key quality features, and more. We have not yet seen the emergence of one-platform-fits-all for the hundreds of clinical trials with AAV. Of the approved products, Luxturna® and Zolgensma® are manufactured using adherent technologies in HEK293 cells, and Roctavian™ and Hemgenix® are produced using baculovirus expression system in suspension in an Sf9 insect cell line.

Q What are your thoughts on transfection optimization?

FV: The transfection step itself is arguably the most critical unit operation to achieve high yield and a high-quality product. For example, this production step sets the baseline for maximizing the number of capsids that contain fully packaged transgene, while minimizing process and product impurities like residual encapsidated host cell DNA and packaging of truncated transgene sequences. To help with this, there are great tools available that allow for the miniaturization of the production bioreactor vessel to test out transfection parameters like transfection reagent and DNA ratios, media compositions and enhancers and other variables. A recently introduced transfection reagent specifically developed for industrial scale production of recombinant AAV in suspension HEK-293 cell types has been shown to boost productivity by over five times. In addition, several tool providers and CDMOs are working on rep/cap plasmid design and helper plasmids to boost productivity and reduce presence of process- and product-related impurities which are exciting ways to control costs by increasing yields and productivity and quality.

DOI: 10.18609/cgti.2023.045

Q How is downstream processing evolving and what needs remain here, particularly in empty-full capsid separation?

FV: A preface to this question is that the upstream production system choice impacts the burden on downstream processing, with producer cell lines and insect cell/baculovirus systems generally providing highest full particles out of production.

When we look at the downstream side, there are many available options for high efficiency and high throughput lysis and clarification, despite vector serotype diversity. Downstream processes should include a virus removal step to remove any potential adventitious agents from the process stream. While it is important to invest time in developing these unit operations with some redundancy to insure against any supply chain issues, the key impact of downstream processing (DSP) is on chromatography purification.

Pan-AAV resins have been developed to significantly simplify purification through the high capacity and high specificity of the ligand. With minimal development and a good elution strategy, you can now achieve consistently high AAV yields with step recoveries near and above 70%. Furthermore, the capture step can be developed to allow direct loading of the clarified harvest, provided you establish load stability, saving materials, time, and cost of manufacturing.

This recent progress shifts the focus to the second chromatography polishing step, where the goal is to enrich full capsids and separate them from empty and partially full capsids. There are two methods to do so in common use: density-based ultracentrifugation (UC), and ion exchange (IEX) chromatography either with resins or other multi-modal media. UC is beneficial because it requires minimal process development, yields high purity vector, and is agnostic to serotype or payload. However, it is difficult to scale, it involves a lot of manual manipulation, and it is difficult to validate. Continuous UC instruments are now being developed which could provide an interesting solution.

On the other hand, IEX requires process development to define product specific parameters. It can be difficult to fully resolve a desired product from multiple empty/partially empty peaks, and it is challenging to achieve the same degree of enrichment as seen with UC. In the future, there is potential for combining IEX with other modes, like hydrophobic interaction chromatography (HIC), to resolve the capsid populations along two axes. There is still much to learn about why empty and partially filled particles elute where they do for different serotypes. Unraveling this could not only unlock better purification strategies, but will also help us understand how to remove these unwanted capsid populations.

As an industry, we have an increasing appreciation that vector distribution is not binary, and we do not yet fully appreciate how these species contribute to our drug product potency. We need further characterization and functional assessments so we can develop more clinically meaningful specifications. In the meantime, our approach will be to establish

purification conditions that result in reproducible elution profiles and consistent product quality and potency with minimal impurities in each run.

Q Which tools and methods will continue to drive development of 'plug and play' vector processes, for you?

FV: Any method or tool that allows our processes to move from the knowledge space into the control space is going to drive plug-and-play. The question is: how to achieve this control? The main levers to pull for standardization are the choice of production system, the efficiency of downstream process for product purification, and the quality assays and methods in place.

Each vector serotype and each gene of interest can have different conditions to optimize and this may even depend on disease indication. Maintaining a consistent output upstream is the first step. Investing time into understanding the behavior of cells before they are placed in the production vessel is also beneficial especially for producer cell lines. The further we move from one-off events by reducing variability, the closer we are to plug-and-play systems – something that pan-AAV affinity ligands have facilitated somewhat. Perhaps a serotype agnostic Anion Exchange Chromatography step or simplified UC would also help drive a 'plug-and-play' approach.

Plug-and-play will allow us to minimize the cost of development across multiple programs, allowing for economies of scale. Efforts such as the Bespoke Gene Therapy consortium, the public-private partnership to establish platforms and standards to speed the development and delivery of customized gene therapies, will contribute significantly to improve vector manufacturing, standardize analytics, and hopefully deepen our understanding of recombinant AAV basic biology.

At the same time, we should be cautious with how we define plug-and-play. As an industry, we are shifting away from the old adage that 'the process is the product'. This to me signals the maturation of our field towards industrialization, and as we move towards true industrialization, we must build products that will be resilient to inevitable process changes, including not only scale changes, raw material changes, and process intensification, but also to potential supply chain challenges. Yes, CGT products are complex, but they are not beyond our comprehension nor our control. To do so, the focus must be on thorough process development and meaningful analytics, in-process and at product release. We must rely on deep characterization, and we can start to incorporate true QbD principles. We may need new tools to be developed, or simply new or improved ways of looking at the data, as we continue to make these powerful medicines with curative potential available to patients.

Q Gene therapy product formulation seems to be of growing concern to the sector – how and where are we improving in this area?

DOI: 10.18609/cgti.2023.045

FV: Formulation is an area where we can invest more effort as an industry. For the most part, we rely on phosphate-buffered solutions with poloxamer and little else. Final product concentration depends on avoiding aggregation and some indications will require higher concentrations than the mid-to-high e[13] vg/mL that is currently the norm. In addition, others have shown that extreme thermal stress results in a loss of biological activity possibly through deamidation.

"..in addition to demonstrating clinical safety and efficacy, we want to achieve maximal accessibility."

Novel formulations that can maintain stability at higher temperatures and shield the product from environmental stress could also be helpful to simplify the supply chain and move away from cold storage, which is often expensive and risky. One potential solution is lyophilization.

Generally, our understanding of how the physical characteristics of the AAV vector impact infectivity and potency is still improving. There is room for significant innovation, as we have seen for other steps of the production process.

Q What are your main priorities for your work over the foreseeable future?

FV: From the viral vector process development side, priorities are those that allow us to achieve robust performance and high-quality product across different serotypes we have in our programs, with an eye to new rapid in-process analytics to inform and direct decisions on the floor and to reduce any future development costs. Our immediate next priority is process intensification solutions and tools with the ultimate goal of reducing the cost of manufacturing. For our multi-component *in vivo* insertion and *ex vivo* drug products, addressing the complexity and cost of virus production would be impactful. Ultimately, in addition to demonstrating clinical safety and efficacy, we want to achieve maximal accessibility.

AFFILIATION

Francesca Vitelli
VP, Cell Therapy and AAV Process Development,
Intellia Therapeutics

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given his approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author has no conflicts of interest.

Funding declaration: The author received financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: This article is based on an interview with Francesca Vitelli carried out on Feb 2 2023.

Interview held: Feb 2 2023; **Revised manuscript received:** Mar 24 2023; **Publication date:** Apr 3 2023

DOI: 10.18609/cgti.2023.045

EXPERT ROUNDTABLE

Driving the expansion of mRNA into the therapeutic sphere

Elisa Manzotti, CEO of BioInsights, speaks to **Alejandro Becerra,** Thermo Fisher Scienitific, **Andreas Kuhn,** BioNTech, and **Metin Kurtoglu,** Cartesian Therapeutics

The advanced therapies industry is heavily engaged in capitalizing upon the extensive 'proof of concept' gained through the success of mRNA-based COVID-19 vaccines. Novel therapeutic applications in major disease areas, including oncology, continue to show promise in preclinical and early clinical studies, yet challenges remain.
Here, a panel of thought-leaders from the mRNA field will consider the ever-expanding reach of mRNA technology, exploring at a high level how and where it will impact the advanced therapies space moving forward. The panel will then dive deeper into specific trends, issues, and innovations in mRNA processing (particularly downstream) and analytical development, discussing key areas for improvement and corresponding solutions.

ALEJANDRO BECERRA is a Principal Applications Scientist and Global Purification Technical Lead. Alejandro has over 15 years of experience in downstream processing and customer support having worked as Purification Team Manager and other bioprocess engineering roles prior to joining Thermo Fisher Scientific in 2018. Dr Becerra is a subject matter expert in preparative chromatography with expertise in the development, optimization and scale-up of antibody, recombinant protein, and viral vector purification processes. Alejandro holds a PhD in Chemical Engineering from Cornell University.

ANDREAS KUHN has worked with RNA for almost 30 years. This started with his diploma and PhD theses on the structure and function of small non-coding RNAs using biochemical and molecular biology methods. In his post-doctoral work, Andreas studied RNA-protein interactions in the spliceosome in yeast and later worked on small molecules to affect pre-mRNA splicing. His work on mRNA-based immunotherapies began in 2007 in the academic group of Ugur Sahin at the University Clinic Mainz, and Andreas joined BioNTech SE shortly after its founding in 2008. In his current role as Senior Vice President RNA Biochemistry & Manufacturing, the main focus is expanding proprietary technologies to increase the efficacy of mRNA-based therapies and to develop and optimize GMP-compatible manufacturing processes and analytical methods for RNA. He has co-authored numerous publications and patents ranging from basic research on RNA to its application as a therapeutic agent and vaccine.

METIN KURTOGLU is a medical oncologist board certified in internal medicine. Dr Kurtoglu's clinical and basic science research career spans over 20 years and has focused on developing novel targets for drug-resistant cancer cells and cancer stem cells, including multiple myeloma. He has also been an investigator in various cancer immunotherapy trials. Cartesian Therapeutics is pioneering RNA cell therapies in and beyond oncology, with three assets in clinical trials for autoimmune, oncologic, and respiratory disorders. The investigational therapies are manufactured at Cartesian's cGMP manufacturing facility.

Cell & Gene Therapy Insights 2023; 9(3), 315–326

DOI: 10.18609/cgti.2023.015

Q What have been the key R&D directions for mRNA and associated technologies, as they expand beyond prophylactic vaccines?

AK: Let me start with stating that while it is often thought that using mRNA was invented for developing the prophylactic vaccine against COVID-19, there had previously been several years of basic and applied research performed with mRNA. This work was the basis for speeding up development of the COVID-19 vaccines.

"When you go after a disease that needs a long-term therapeutic effect, it will be challenging to produce the right type of RNA in a formulation that results in sustained therapeutic activity."

– **Metin Kurtoglu**

A related approach to mRNA-based infectious disease vaccines is using mRNA to activate the immune system to kill cancer cells in a therapeutic setting. With the same goal, mRNAs are also used to encode proteins such as antibodies or cytokines, which for me, is a key application area. On the research side, there are two main areas of development: firstly, the mRNA molecule itself, and secondly, the formulation, which is where lipid nanoparticles come in. More development in formulation is needed, as it will be key for the field to move beyond the liver into other tissues – intramuscular delivery for the prophylactic vaccines was very important.

While there are already efficacious vaccines on the market, there is an opportunity to further improve the mRNA molecule itself – for example, lowering dose and improving tolerability are two key areas if we are to successfully move beyond prophylactic vaccines.

MK: Another exciting current event in the field is the expansion of mRNA therapeutics into new indications. Because mRNA is now used for vaccination purposes on a global level, people start to get more comfortable with using mRNA elsewhere – for example, in autoimmune disorders or other internal medicine diseases. Secondly, in terms of mRNA delivery, one very interesting solution in development is delivering mRNA therapeutics in the context of a live cell. In both autologous and allogeneic cell therapy areas, there are some very interesting new technologies going after unique diseases using live cells transfected with mRNA.

AB: Being a supplier of products for the manufacturing of RNA, we are in a position where we can look across a wide range of both smaller and larger organizations and see what they are working on. We observe the movement not only towards vaccines but also to personalized medicine, such as cancer vaccines in the oncology space. With regards to the mRNA itself, there is a big focus on utilizing different approaches to reduce dose, as Andreas mentioned – whether it is with traditional mRNA, self-amplifying RNA, or new molecules like circular RNA (circRNA).

Q What are some of the major challenges that face the field as it makes this migration into therapeutic drug applications?

"Being more efficient in delivery and formulation is a major challenge the field needs to overcome if it is to open up all the opportunities which mRNA as therapeutic modality has to offer."

– **Andreas Kuhn**

MK: Using mRNA to vaccinate against infectious diseases works really well. The mRNA itself is very immunogenic and the body will immediately react to it. However, when you go after a disease that needs a long-term therapeutic effect, it will be challenging to produce the right type of RNA in a formulation that results in sustained therapeutic activity. The greatest challenge in moving beyond infectious diseases and into the therapeutic sphere is to come up with less immunogenic solutions. At Cartesian, we are focused on using live cells as a vehicle because the cells protect the mRNA within its physiological environment, reducing immunogenicity.

AB: From the development perspective the purity of the mRNA is critical, and closely associated with purity are the analytical challenges. A purification process is only going to be as robust as the analytics that are available to develop it. It will be critical to establish better methods in order to characterize the product-related impurities. Next to this technical aspect, another challenge in continuing to develop technology is having the knowledge and expertise available in all parts of the world – building a skilled workforce with the requisite training provision is important.

AK: Less immunogenic and purer mRNA is not going to be sufficient for delivering success in advanced therapy applications. With vaccines, only relatively small amounts of protein are needed in order to obtain a huge amplification by the immune system. On the other hand, using mRNA for the expression of functional protein requires several orders of magnitude higher expression of that protein. Therefore, looking into improved expression of the mRNA is key – for example, through improved sequence design.

In addition, I would like to reiterate the importance of delivery. With the current methodologies, it is often that only a small amount of the injected mRNA ends up in the target cells. Being more efficient in delivery and formulation is another major challenge the field needs to overcome if it is to open up all the opportunities which mRNA as a therapeutic modality has to offer.

Q What will be the key technological/platform developments and innovations required to address these challenges?

DOI: 10.18609/cgti.2023.015

"The ongoing efforts and collaboration between suppliers and producers of mRNA therapeutics will lead to the development of new products and will help accelerate production processes."

– **Alejandro Becerra**

AB: There are ongoing efforts to improve the purification toolkit for the mRNA field. More specifically, when we are looking at eliminating double-stranded RNA (dsRNA) from the final product, current efforts are focusing both on the *in vitro* transcription (IVT) reaction as well as the downstream process. There are some potential approaches that might be difficult to scale up today, but with the ongoing efforts and collaboration between suppliers and producers of these therapeutics, it will lead to the development of new products and accelerate production processes.

AK: We still have a lot to learn about mRNA. The key is to understand what makes a specific mRNA optimal in the context of using it as an exogenous mRNA. To build more knowledge on bringing mRNA into the cell, we need to understand what happens with the mRNA: how is it taken up by the cells? What factors in the cells are important to translate the mRNA?

Another development is taking some of the technologies that are used for other biological molecules and applying them to mRNA. There is a lot of existing knowledge on purifying biological molecules, including on the analytics side, that can be applied to mRNA. We will need improved analytical techniques to better understand what the molecule is that we have in hand.

MK: Hopefully, the number of products in the RNA cell therapy world will start to expand once we start showing promising data in more indications using therapeutic RNA in the context of a cell. The cell uses the mRNA for the therapeutic function at the right level, because these cells intrinsically know how to express at a level that causes bioactivity. These products are just starting to enter clinical trials and as they are beginning to show proof of concept, we will see more live cell therapy applications using RNA. In this way, the therapeutic RNA activity can be taken to organs by using their physiological pathways, rather than trying to figure out all the details exogenously.

Q Looking at mRNA therapeutic manufacturing, what are the main limitations with the current processing tools and technologies? Where specifically do we need to improve in both upstream and downstream processes, and what approaches will yield this progress?

AK: As we have discussed previously, purification is one area where further improvements can be made. mRNA are highly charged molecules, which causes issues with some of the more standard types of chromatography such as ion exchange chromatography (IEX). New tools are needed to help us to purify mRNA at larger scales – not the micrograms to milligrams of mRNA required for preclinical proof-of-concept studies, but tens to hundreds of grams.

Looking at the IVT reaction itself, T7 RNA polymerase is a well-behaved enzyme in general, but it has some limitations such as creating double-stranded RNA as a byproduct. Developing a broader toolbox with alternative RNA polymerases that have better characteristics will be useful. If the product coming out of the IVT reaction is purer it will put less stress on the purification process.

Another current limitation with the materials is the need to renew the DNA template whenever you produce a new RNA sequence. New technologies to assemble and amplify DNA – for example, in a cell-free process rather than using *E. coli* – would be helpful in quickly getting a DNA template for mRNA manufacturing.

AB: When we look at how manufacturing tools have evolved for other biologics such as monoclonal antibodies, some of the more important advances came through collaborations between manufacturers and suppliers. We should leverage a similar approach in the mRNA field and work on close collaborations to develop the new tools. These joint efforts will get us to the right tools faster.

MK: The design of the mRNA is the biggest challenge in mRNA manufacturing. How much mRNA is needed to make enough protein in order to achieve the therapeutic function? The answer is that the amount of mRNA required depends greatly on the design of the mRNA. If you can design a mRNA where you only need a microgram to give the desired therapeutic effect, then manufacturing is no longer going to be a challenge. The second challenge relates to the delivery system: whether you are a LNP or a cell, the limitation and bottleneck right now is in scaling up of the delivery systems.

Q Can you provide insight into the current practice of process monitoring and optimization of IVT and LNP formulation?

AK: At this point there is to my knowledge no online process monitoring available for mRNA manufacturing. There are some tools for reporter constructs that have a fluorophore sequence element in the RNA, but this is very specific and can only be used to perform generic process development. Right now, we look at the reaction over time by taking samples and monitoring the effect, which is a laborious practice. At some point, when the technology is mature, tools will become available to monitor the productivity and yield of the RNA production process online. Hopefully, we will move into the sort of online monitoring

DOI: 10.18609/cgti.2023.015

that we see today in fermentation processes, where one can respond rapidly and add nutrients when the cells are growing too slowly, for example.

Q Can you go deeper on how the mRNA purification toolkit is evolving to address current challenges?

AB: The main unit operations in purification are the filtration and chromatography steps. We see a significant number of manufacturers utilizing POROS™ Oligo(dT) affinity resins for one of the chromatography steps in the process, whether it is after the IVT and/or after the capping (when the capping takes place post-transcriptionally). In terms of filtration, there can be multiple tangential flow filtration (TFF) steps and of course, a membrane filtration step at the end.

The currently available filtration toolkit can be improved. For example, particularly for smaller companies that are just starting to develop their process, there might be instances where there is a lack of a representative scale-down model. For chromatography and more specifically, with POROS Oligo(dT) affinity resin, I think we are in a good place today, but with the need to purify larger molecules, the binding capacities can be relatively lower. At the moment, Thermo Fisher Scientific is looking into collaborations to investigate different strategies to maximize the binding capacity with commercially available products. Long-term, we are looking into developing more specific base beads or other chromatography supports that can further improve the performance.

The polishing steps have challenges as well – for example, when reversed-phase chromatography is used and there is a need to utilize solvents (particularly an issue at larger scales). There is room for improvement, whether it is through new chemistries, or different approaches and methods that are more good manufacturing practice friendly.

MK: To make an incredibly pure mRNA is always a challenge. One way to circumvent this challenge is to allow some impurities in your therapeutic product that will not impact the outcome whether it relates to safety or efficacy. Choosing a cell as the delivery vehicle will help here, because the cell has built-in mechanisms to eliminate impurities such as nucleoside triphosphates (NTPs) or double-stranded RNA. More interestingly, you could manufacture a cell in such a way as to ensure those impurities are eliminated by the cell during manufacturing.

AK: One thing that can help overcome the lower capacity challenge of chromatography resins is to establish a form of continuous chromatography. Rather than having to increase the column size in relation to your batch size, you could overcome the lower binding capacity challenge by prolonging the process.

Q What are the key areas for improvement in the analytical toolkit?

MK: mRNA is a fairly heterogeneous molecule by nature. For example, the length of polyA is usually not uniform. Finding the right analytical tests to determine the features of these heterogeneous mRNA molecules is challenging.

AK: One of the challenges at this moment is the diversity of methods used to analyze the same parameter. One example is measuring RNA integrity, which indicates the amount of full-length RNA versus the amount of degradation products or truncated transcripts. Analysis of RNA integrity can be performed by using a large variety of techniques and you can question how the results of these different techniques correspond to each other. Harmonization and standardization of analytics is very important for moving forward. A comparable situation is the use of internal standards to measure double-stranded mRNA. Individual companies are using different standards at the moment, which raises the question of how comparable the numbers are.

On the other hand, there is the challenge of the technical limitations that some of the analytics have. Developments are taking place – for example, in the sequencing technology area – that will help us to better analyze samples. More advanced sequencing technologies are emerging, which can improve knowledge of the mRNA molecule itself.

Q Do you expect regulatory guidelines to be set for mRNA and siRNA manufacturing in the near future? If so, how do you see these guidelines impacting the freedom to operate that the field enjoys today?

AK: The European Directorate for the Quality of Medicines (EDQM) has started an initiative to draft guidelines for mRNA therapeutics. Initially, it will be for prophylactic vaccines because these products are already on the market, but this initiative will definitely help in creating guidelines for all mRNA therapeutics. There is already a guideline from the World Health Organization (WHO) and the US Pharmacopeia (USP) has drafted a guidance document as well. Most likely, there will be more to come.

The question around freedom to operate is interesting… When there are no guidelines, people complain that they don't know what to do, but when there are guidelines, they complain that they have to follow them! At the end of the day, regulators usually have good reasons why they ask for certain things, and more regulatory guidelines will clearly help further development of mRNA therapeutics.

MK: Having regulations that are outdated and restrictive is even worse than having none at all. In the field of mRNA that is changing now, due to the COVID vaccines. People have started to differentiate regulations for mRNA from DNA. This is key because all the guidance that existed before stemmed from DNA-based therapies. However, unlike DNA, mRNA is a biologically degradable molecule. DNA lives for years – millions of years, in some cases – but that is not the case with mRNA. Thus, the safety profiles for mRNA are

DOI: 10.18609/cgti.2023.015

a lot stronger than those of DNA-based approaches (which is why allowing certain impurities in mRNA therapeutics may be acceptable, if it does not impact the efficacy). Nevertheless, historically speaking, all the regulations, guidelines and analytical tools have been focused on DNA-based engineering, solely because that technology has been around longer in terms of therapeutic applications. But regulatory guidelines for mRNAs are catching up.

Additionally, the way in which you analyze mRNA that is delivered in a living cell will be very different than how you analyze mRNA that is administered through LNPs. Regulatory guidance will evolve over the next few years as more products using different RNA-based approaches come out. The guidance will need to be finetuned depending on the specific product.

Q Concerning the optimization of current mRNA-LNP formulations, does the panel have any ideas on what is the preferred target in this regard?

AK: If you go beyond vaccines you are going to have different cells that you want to target. In a case where mRNA is used in protein or transcript replacement therapy, when there is a protein missing due to a genetic defect, you must get the mRNA into that specific cell type. The question is, what cells can we reach? The more different formulations you have that can deliver the mRNA to a specific cell type, the more diseases you can tackle. Some cell types will be easier to target. If the field goes beyond and into the brain, for instance, then it will be necessary to find something able to cross the blood–brain barrier, which will be a lot more challenging.

Q What do you see as the most challenging step in the downstream process?

AB: One of the bigger challenges we see, particularly for mRNA therapeutics as opposed to vaccines, is the removal of double-stranded RNA. There has been some success with reversed-phase chromatography, but with the inherent challenges of scalability and using high temperature solvents that we discussed previously. Fortunately, this challenge may also be addressed during the IVT process, so it will hopefully be solved in due course.

Q Does Thermo Fisher manufacture any other bead-based products for purification of mRNA apart from the POROS Oligo(dT) Resin?

AB: We mentioned that there are different approaches and it all depends on the required purity of the initial material. When the final use of the mRNA is as a vaccine, then affinity purification and filtration may be sufficient. But where you need to remove double-stranded RNA and other product-related impurities, you will need alternative methods

such as reversed-phase, ion exchange, and/or hydrophobic interaction chromatography. Thermo Fisher does offer these alternatives. With the latter two, we are still learning together with our customers about whether they will be the right tools for this particular purpose.

Q Finally, can you sum up your visions for how and where mRNA will impact across the advanced therapies field in the future?

AK: Due to the success of the development of the mRNA-based vaccines against the coronavirus, expectations are high right now. As we see with many new technologies, people are overestimating the short-term benefits and then underestimating the long-term effects. It will take time to have the next mRNA product on the market and there will probably be some drawbacks and challenges that the field needs to overcome first. In the situation with the coronavirus, we knew which protein to tackle. The formulation was there, the mRNA was there.

Ultimately, though, mRNA will have a huge impact on medicine in general. Similar to where antibodies are today, mRNA therapies will make up a huge part of the market. The next mRNA products on the market will most likely be prophylactic vaccines to fight other infectious diseases such as influenza, RSV, malaria, and HIV to name a few. The second wave will be in oncology products, especially where similar approaches can be taken as with the vaccines, meaning stimulating the immune system to attack cancer cells. There is promising data coming out already here, especially with individualized approaches where cancer cells are sequenced, and you identify new epitopes that are very specific to the cancer cell. After that, I would say the next breakthrough will either be new therapies to battle genetic diseases, or in the field of antibody- or cytokine-encoding mRNAs.

AB: We may see some of the first new products coming out in the vaccine space. Hopefully, other geographies will have access to these new vaccines, and the focus of these vaccines might be on diseases that are more prevalent in other parts of the world, beyond North America and Europe. In the long-term, we will see more growth in *ex vivo* or gene editing applications of mRNA, in addition to more therapeutic application areas such as monoclonal antibodies.

MK: Managing expectations is important. If you want to go after infectious disease vaccination, mRNAs are immunogenic and work great. The gap to the next chapter for mRNA therapeutic applications is large. The good news is that people have been working on RNA therapeutics since the field was invented in 1970s, and have invested heavily since the 1990s. Hopefully, these 30–40 years of development have given us new insights that will help to close the gap faster. Still, the fact that there has been 30 years of work done on mRNA and yet no product other than COVID-19 vaccines came to market, does points to developing RNA based therapeutics is a challenging task. I am hopeful, though, that new applications will come on the horizon in less than 5 years. I think that what can happen quickly is combination

DOI: 10.18609/cgti.2023.015

therapy through mRNA vaccination, where you will be able to vaccinate people against multiple diseases simultaneously.

There are some new and unique indications in oncology and there are new programs in autoimmune disorders, a completely new field that RNA therapeutics never reached before. There might be unexpected therapeutic indications that come up, too, because mRNA is a very versatile tool. There is going to be a greater explosion in mRNA therapeutics than will be in the DNA-based engineering field over the next 5–10 years.

AFFILIATIONS

Alejandro Becerra, PhD
Thermo Fisher Scienitific

Andreas Kuhn
BioNTech

Metin Kurtoglu, PhD
Cartesian Therapeutics

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Kuhn A discloses he has royalities/licenses, receives support for travel/meetings, patents planned, issued or pending and stock/stock options from BioNTech SE. Kurtoglu M discloses he has patents planned, issued or pending and stocks/stoc options from Cartesian Therapeutics. The other author has no conflicts of interest.

Funding declaration: Kuhn A received financial support for the research, authorship and/or publication of this article from BioNTech SE. Kurtoglu M received financial support for the research, authorship and/or publication of this article from Cartesian Therapeutics.

ARTICLE & COPYRIGHT INFORMATION

Article source: This article is based on a expert roundtable webinar, which can be found here.

Expert Roundtable webinar conducted on: Feb 23 2022; **Revised manuscript received:** Apr 6 2023;

Publication date: Apr 20 2023.

We hope you enjoyed this transcript of the roundtable
You can also watch the recorded roundtable here:

WATCH NOW

ThermoFisher SCIENTIFIC

Helping you find your way to improved mRNA purity and yield

Simplify your mRNA purification process

POROS™ Oligo (dT)25 Affinity Resin is designed for the purification and isolation of mRNA from *in vitro* transcription (IVT) manufacturing processes. The use of affinity chromatography offers high selectivity and ease of use. Through the AT-base pairing mechanism the resin effectively separates mRNA from components of the transcription reaction process, such as enzymes, unreacted nucleotides, partial transcripts and plasmid DNA.

Why choose the Oligo (dT)25 affinity resin?

- Easy mRNA purification from crude transcription mix
- High dynamic binding capacity and high recovery
- Simplified workflow helps to maximize efficiency and reduce the complexity of subsequent polish steps
- Excellent scalability, allowing purification from benchtop to commercial manufacturing

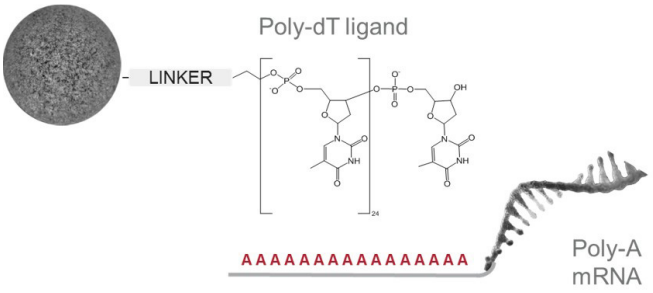


Figure 1. Mechanism of action of POROS Oligo dT(25) affinity resin. The poly-dT ligand allows binding with poly-A tailed mRNA molecules through AT base pairing.

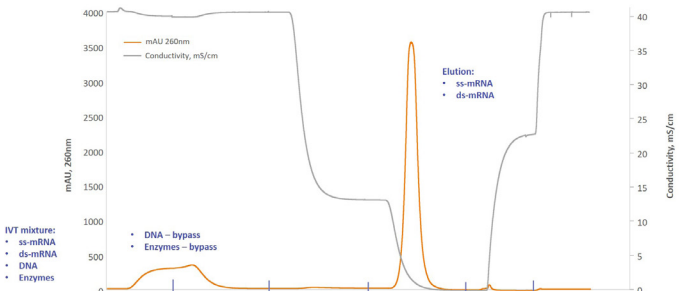


Figure 2. Chromatogram showing efficient separation of a 2000nt mRNA from an IVT mixture at a load concentration of 2 mg/mL. Elution was performed using H_20 and yielded >95% recovery.

Discover more at **thermofisher.com/oligodT25**

thermo scientific

COL34657 0422

CELL & GENE THERAPY INSIGHTS

VECTOR BIOPROCESSING & MATERIALS

SPOTLIGHT

INNOVATOR INSIGHT

Sustainable plasmid DNA strategies, achieving streamlined secure supply to clinic & commercialization

Andrew Frazer & Amanda Weiss

With many advanced therapy products reaching commercialization and an ever-increasing pipeline planned for transition to late-phase clinical studies, there is significant demand for reliable plasmid DNA supply. In addition, evolving and sometimes undefined regulatory requirements and quality standards present a range of opportunities and challenges for developers. This article will explore some of the common challenges encountered when sourcing plasmid DNA, and provide valuable recommendations that will help navigate the pitfalls in achieving sustainable plasmid supply to support clinical programs through to market. A case study will highlight key lessons learned to help developers set themselves up for success when working with CDMOs.

Cell & Gene Therapy Insights 2023; 9(3), 379–388

DOI: 10.18609/cgti.2023.058

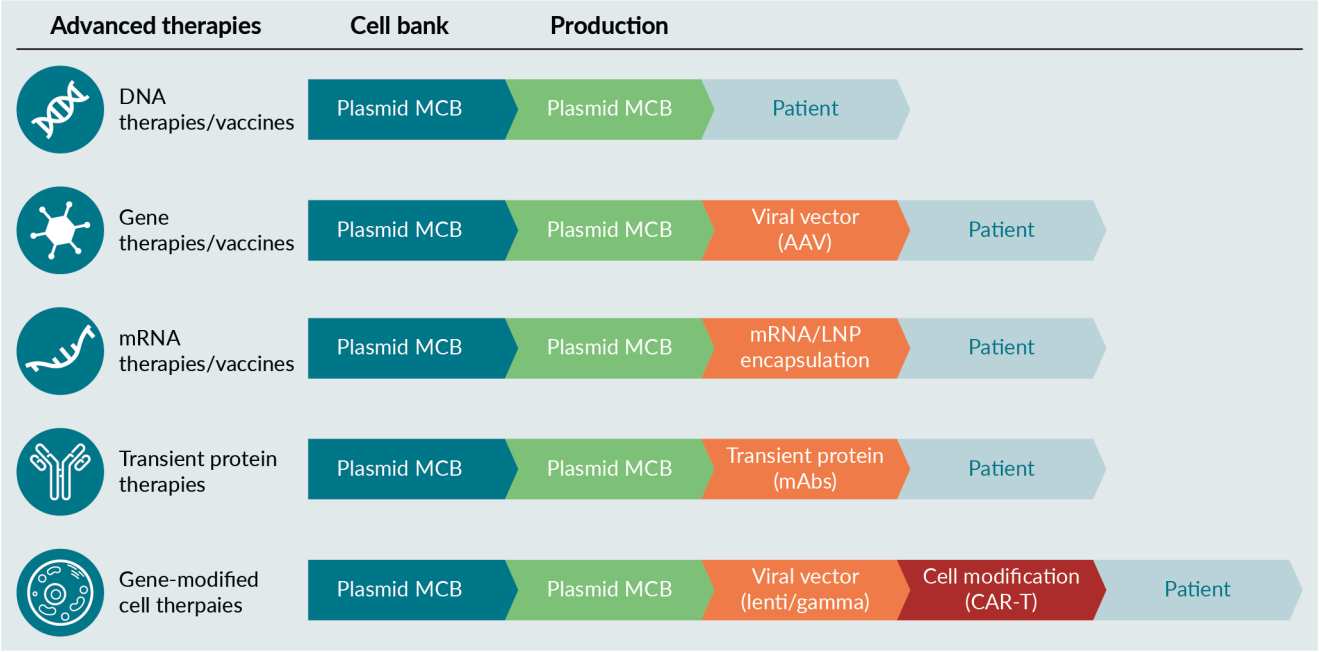
PLASMID DNA

Plasmid DNA (pDNA) plays an increasingly important role in modern healthcare strategy. The global pDNA manufacturing market is significant, with an estimated value of $400–700 million in 2022 and a projected compound annual growth rate of >20%. The expanding R&D pipeline of advanced therapies is a key factor in this growth with any advanced therapy approaches relying on pDNA as a critical starting material or as a direct therapeutic product in the case of naked DNA therapies or plasmid vaccines (Figure 1). More specifically, the use of pDNA as a critical starting material in transient transfection-based viral vector production is driving increased demand, and this in turn is creating challenges and opportunities for both suppliers and consumers.

In the development and delivery of a medicinal product, the building blocks rely heavily on what precedes them, so it is critical to have a safe, reliable, and cost-effective

FIGURE 1

Use of pDNA in advanced therapies as a drug product or as critical starting material.



supply of starting material to deliver effective treatments to patients.

ADDRESSING COMMON PLASMID SUPPLY CHALLENGES: THE EXPDNA™ PLATFORM

Charles River's recently launched eXpDNA platform has been established to address some of the most common plasmid supply challenges, to deliver improved timelines with a focus on process and product quality attributes. The eXpDNA platform has been developed based on three core capabilities. The first of these is a 'plug-and-play' toolbox approach to enable the adoption of various levels of screening and quality control checks. This mitigates the manufacturing risks often associated with complex and challenging plasmid types, which are commonly used for advanced therapy applications. The second is a standardized platform manufacturing process allowing for a streamlined supply chain and documentation management. Thirdly, the platform offers the ability to implement 100% in-house analytics for both cell banking and final product release testing. By integrating these three capabilities, industry-leading batch timelines are delivered whilst maintaining product quality.

The plug-and-play development toolbox plays a key role within the platform and following an initial technical assessment of the plasmid sequence, the Charles River Laboratories team will identify the type of construct and any key features that are critical to its function. This assessment informs the approach for the subsequent cell banking steps and dictates which additional screening or quality controls are included to ensure a smooth progression through manufacturing, testing, and release. There are many plasmids that can progress directly through manufacture with minimal checks or screening however, some of the more challenging types (e.g. inverted terminal repeat (ITRs) containing gene of interest (GOI) plasmids for AAV, or mRNA template plasmids containing long poly(A) sequences) typically involve extra steps to mitigate their unstable nature and propensity for recombination during cell banking and manufacture.

The Ambr250 system is an effective tool for early-stage screening and pre-production

DOI: 10.18609/cgti.2023.057

CASE STUDY

Securing plasmid supply for kidney disease gene therapy.

Purespring Therapeutics is the first gene therapy company with a unique platform and delivery system for local administration to the kidney. This system targets the kidney podocyte cell, leveraging science from co-founder Professor Moin Saleem of the University of Bristol. The company has a research and development pipeline of three programs, with a discovery engine powered by state-of-the-art search engine, FunSel. Purespring Therapeutics was established in 2020 through funding from Syncona Partners with the aim of building a sustainable and global commercial organization.

Kidney disease is a huge unmet medical need. Roughly one in eight people around the world suffer from chronic kidney disease and by 2040, it is predicted that the number of deaths from kidney disease could be more than three million people a year worldwide. Purespring Therapeutics is developing a local delivery method approach to treat kidney disease that targets the kidney directly, reducing dose and limiting systemic exposure. This will be supported by a proprietary procedure.

The podocyte is a terminally differentiated cell in the kidney, making it an ideal target for gene therapy. As part of the glomerulus, it has several functions including regulation of filtration, maintenance of renal architecture, and regulation of complement modulation. The podocyte is implicated in approximately 60% of renal diseases - this wide-reaching implication in several diseases was a key reason why it was chosen as a platform target. This platform approach across various indications enables the use of the same plasmids (a rep/cap and a promoter) and targeted route of administration, which results in lower risk, reduced cost of goods (COGs), and accelerated development timelines. Analytical development and CMC strategy also benefit.

A triple transient transfection process into serum-free suspension cell culture is used for the AAV upstream process. Plasmid quality is important, as a consistent, high-quality feedstock allows the development of a robust and reliable process. CMC principles are adopted to safeguard future development and reduce delivery risk. It is important to anticipate the regulatory environment when outsourcing plasmid supply. Having the same source of plasmids for both preclinical R&D and first-in-human trials will lead to fewer potential changes in future. Charles River Laboratories was selected as Purespring's plasmid supplier based on the fulfillment of several key criteria, which include offering fully GMP-compliant plasmid, a comprehensive quality system, and the ability to release plasmid directly. Good capacity and speed were further considerations, in addition to having a supportive and flexible team. The partnership is open, interactive, and has demonstrated success.

At Purespring, a number of principles are used to help de-risk partnerships. These principles include a solid foundation, a close regulatory and quality fit, and the ability to leverage technology platforms. The collaboration with Charles River reflects these principles, with GMP-compliant quality and flexibility helping to further future-proof the investment.

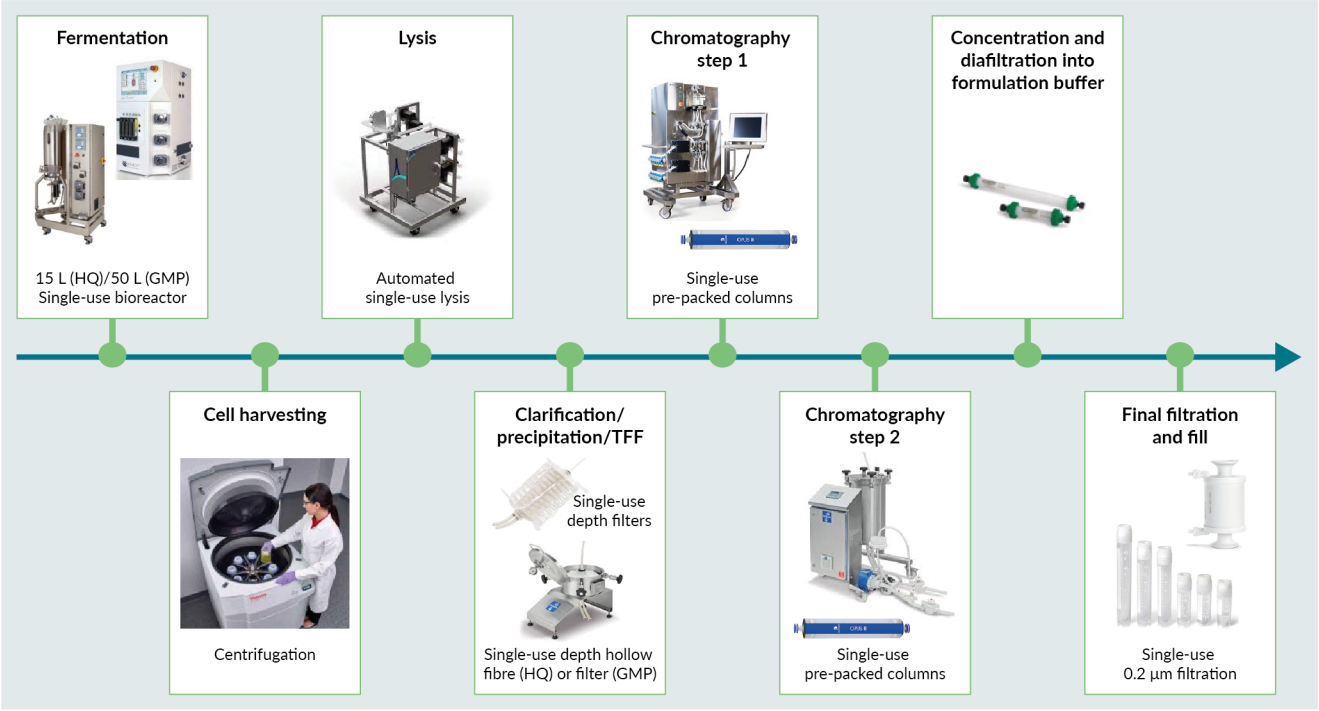
evaluation. This is used routinely to provide an early indication of process performance and yield prediction. This type of assessment can be implemented on its own, but also in parallel with pre-banking and clone selection steps to confirm plasmid integrity using methods such as next-generation sequencing or genetic stability testing. The utilization of one or both options has a strong track record in managing challenging plasmid types and can be implemented with relatively little impact on overall project cost and timelines.

To support eXpDNA platform deliverables, the plasmid production process utilizes fully single-use materials and process flow to accelerate timelines and ensure greater quality control **(Figure 2)**. The process is not plasmid-specific, but instead has been designed to accommodate a wide range of different plasmid types without the requirement to perform extensive and time-consuming optimization work. Both main production processes for high quality (HQ) and GMP plasmid service offerings now operate with a dedicated, fully single-use process stream to deliver predictable scale-up and reliable product specifications in addition to retention of stable plasmids through the full manufacturing process.

Single-use processing provides several benefits. When operating multi-product facilities with the parallel manufacture of a range of different plasmid products for different end users, one of the main advantages is the avoidance of time-consuming equipment cleaning and residuals testing between batches. The fully single-use process stream also effectively

FIGURE 2

The Charles River pDNA manufacturing process.



rules out the possibility of product cross-contamination when combined with established inter-batch cleaning procedures.

Whilst there are clear advantages to single-use processing, those involved in the bioprocessing industry will have experienced challenges around the supply of single-use components, including long lead times for bioreactor bags and tubing manifolds. Suppliers of single-use products are making their own improvements and lead times are improving however, Charles River Laboratories recognized this issue and its impact on the ability to initiate and delivery manufacturing activities. Significant effort has been made into standardizing processes and materials, and into leveraging partnerships with global suppliers to support requirements for the eXpDNA platform. This has enabled simplification of the supply chain and furthermore, holding larger stocks of materials effectively allowing immediate initiation of manufacturing projects.

The eXpDNA platform offers a range of quality standards to support all stages of clinical development. HQ-grade plasmid represents a cost-effective step-up in quality from R&D-grade plasmid, offering a phase appropriate alternative to full GMP manufacture. While often used at an early stage, it can also be adopted in some applications for toxicology studies, as a critical starting material for GMP vector production in Phase 1 or 2 clinical development, or as a template for GMP mRNA production. HQ-grade plasmid manufacturing incorporates a range of GMP principles and uses fully traceable materials, comes with a comprehensive documentation package, and is produced in dedicated HQ production suites with delivery timeframes for custom plasmid products of as little as five weeks. GMP products are manufactured to the highest possible standards in a licensed GMP production facility with fully comprehensive documentation, testing, and quality assurance (QA) oversight. The GMP plasmid is the gold standard and can be used at all stages of development and commercial manufacture of viral vectors and DNA vaccines. Due to the advancements within the new eXpDNA platform, GMP plasmids can be delivered in ten weeks.

DOI: 10.18609/cgti.2023.057

With the eXpDNA platform, customers have the option to implement any of the available screening tools within each quality grade to ensure reliable production. However, when investing in upfront work to future-proof plasmid products and supply, it is important to understand long term requirements and limitations, particularly in the choice of cell bank quality.

The quickest and cheapest option is to establish a research cell bank followed by a research-grade plasmid, but it is important to recognize that it is not possible to directly use research cell banks for HQ- or GMP-grade plasmid manufacture. The transition later on to increase quality grades can be challenging with regulators and the additional manufacturing cost and the time to establish new cell banks need to be factored in and considered for longer-term supply requirements.

Adoption of HQ-grade production cell banks provides a middle ground and developers can realize some cost and time savings vs. GMP grade MCBs. There are scenarios where the use of production cell banks and HQ-grade plasmid can satisfy supply requirements through to commercial, though there is still the ongoing risk that regulators will tighten up on requirements and developers will then need to go back and establish a GMP-compliant supply. A larger number of customers are now investing in GMP-grade master cell banks at an early stage to provide high levels of flexibility in the longer term while still retaining the option to transition easily over to R&D plasmid supply if needed. It gives a strong foundation of quality at an early stage and future-proofs supply, mitigating the risk of any future regulatory changes or of having to incur comparability studies that could have major impacts on the time and cost of advanced therapy programs.

Q&A

Andrew Frazer, Associate Director of Scientific Solutions, Charles River Laboratories (left) and **Amanda Weiss,** VP CMC, Purespring Therapeutics (right)

Q How does choosing a master cell bank (MCB) over a primary cell bank (PCB) impact time to clinic?

AF: First, it is important to appreciate that there are many contributing factors that influence plasmid supply options for different companies depending on stage,

funding, time, and cost. It is important to understand the options and plan ahead. If a customer knows early on that they will at some point need to make the transition from HQ- to GMP-grade plasmid, we recommend choosing an MCB as early as possible, as that can be used to produce HQ-grade material or even R&D-grade material easily versus a PCB. In addition, avoiding comparability studies and the additional manufacture of an MCB at a later date is significant with regard to long-term project timeline and cost.

AW: One of the biggest hurdles with any contractor is signing a Master Service Agreement (MSA) and quality agreement, which can take some time. With respect to speed, a GMP cell bank does take longer to manufacture, but having a consistent starting material that can feed the production of your R&D-grade plasmid and then GMP plasmid is useful. When making an R&D cell bank (particularly with a GOI) if we were to do a new transformation in GMP, the GMP material you generate could be different from the material you generate from your R&D cell bank. Making your master cell bank early and using that as the source of all your starting material helps future-proof your process and the plasmid being used for development and clinical studies.

Q What additional reasons did you have for choosing the GMP-grade plasmids for your first-in-human studies over HQ-grade?

AW: We chose GMP-grade because we use that in our toxicity study. The regulators do not necessarily stipulate that as a need, but because we want our toxicity readouts to be indicative of what the human studies may or may not look like, and ensure safety, we chose GMP-grade. Being consistent and having those same starting materials throughout was key for us. If something goes wrong or changes, then we want to rule out our starting material as a contributor to an adverse event.

Q Do you foresee a continued demand for 'Principles of GMP' plasmids, or an increase in cost pressures on full GMP-grade?

AF: There does seem to be an increase in the level of testing and quality control standards around HQ-grade plasmids. Regulatory advice, such as that from the European Medicines Agency (EMA) on the principles of GMP, gives manufacturers a much better basis to plan and deliver on manufacturing activities. With regards to application, if we see a continual tightening of regulations around testing and manufacturing, it might eventually transition to GMP being used as standard, with intermediate grades like HQ being phased out. GMP plasmid is expensive, and one of the challenges with advanced therapies is their cost. Having complex manufacturing approaches and materials involved in the delivery of these therapies does contribute heavily to those costs. At Charles River, we do a lot of work on reducing the COGs. Having the ability to utilize a phase-appropriate supply like HQ-grade and using prin-

ciples of GMP to risk-assess the potential impact on patients also helps to drive down timelines and costs. If we see wider adoption and acceptance by regulators of products where HQ-grades are going through to late-stage clinic and even into commercial supply, then we could see it continue for a long time.

Q What is your perspective on whether there is a movement towards an actual regulatory requirement for GMP rather than HQ?

AW: If more people use GMP-grade as critical starting materials, regulators could adopt those practices as they like to see the highest standard possible. The reason we do it is to future-proof those processes and although it is expensive, if we end up requiring a comparability exercise, it can be cheaper overall, particularly in a rare disease indication.

AF: There is a regulatory grey area around some of these intermediate products. The additional guidance is helping, but it is application-dependent for AAV. The vast majority of our customers are moving to GMP when they get into the clinic. In cell therapy applications where plasmid is a critical starting material, and there are multiple GMP manufacturing steps and product release between plasmid CSM and the patient, we may see longer-term applications of HQ-grade DNA.

Q What are the real-world advantages of being a single-source provider?

AF: Within Charles River, we are moving quickly towards the goal of being a single-source provider. Bringing on these new CDMO manufacturing sites has added a big piece to the jigsaw. For advanced therapies, there is published data to show that there is an increase in issues and complications with CMC and much higher disruption for regulatory approval versus more traditional products like monoclonal antibodies. We feel that an integrated product approach from a single-source provider can help delivery by increasing process efficiency. The eXpDNA platform allows effective and timely decision-making. Utilizing a single experienced and multi-disciplinary team supports the effective use of data for project progression and problem-solving. One of the most important things to do is to align your manufacturing timelines and testing with study activities to optimize scheduling. It is difficult to give a concise answer to the real-world advantages, as there is huge opportunity and scope to improve in this regard.

AW: It helps the client out because if you have your AAV manufacturer looking after your plasmid supply, they need to ensure that they have plasmid readily available to start AAV manufacture. It helps with logistics, timing, and release of those critical starting materials into your vector supply. As a small business who outsources a lot of activities, putting these aspects together helps with the management and logistics of plasmid supply.

Q Besides lead time, what are some of the other advantages of off-the-shelf plasmids?

AF: Lead time is a key benefit but from a manufacturing point of view, having some of these commonly used plasmid types available helps reduce pressure on our manufacturing and testing capacity. Being able to generate these plasmids in larger bulk quantities means you receive the advantages of economy of scale, allowing prices to be reduced. Customers can plan ahead and place orders for their programs on a gram basis versus on a batch basis. With plasmids, it is typical to see variability around batch-to-batch yields, and being able to order quickly on a gram basis would help with avoiding under/overordering and wastage. There are also many secondary benefits to a reduction in supply chain complexity. If we can standardize the use of these common packaging and helper plasmids, then it has the potential to ease regulatory approval and introduce follow-on pipeline products for developers in the longer term.

AW: As with any raw material or active pharmaceutical ingredient (API) product, you have to put plasmid on stability testing. Our bespoke plasmids are all on stability, which means you have to sacrifice an element of your batch to stability testing. For me, being able to buy plasmid (and particularly things like helper plasmid) off-the-shelf is useful, as I would then simply refer to Charles River's Drug Master File (DMF) as part of our regulatory filing with the manufacturer. That supports the stability and the storage conditions of those plasmids sufficiently, which saves me time and money.

Q How are you defining the specific differences between the research cell bank (RCB) and PCB? Is there testing or documentation?

AF: There are some key differences, including documentation and testing. In terms of documentation for RCBs, we generally work from a manufacturing protocol, not a batch manufacturing record as you would see for an HQ or GMP process. The level of quality oversight is much lower compared to HQ or GMP. Testing panels are typically heavily reduced. Usually, customers can request to have any of our GMP tests, including for RCB, but typically, they are not interested because the material is not high value, and their level of application does not require much coverage. At Charles River, we use the same assays, so our customers benefit from this continuity: if they want to transition upwards in the quality grades, the testing and compatibility studies will be easy.

The manufacturing environment is likely the biggest difference. For research-grade, manufacture is done in the research laboratory, with minimal control of the environment. There would not be the same process and procedures that you would have for HQ or GMP.

BIOGRAPHIES

ANDREW FRAZER has attained over 10 years of experience in the design, implementation, and tech-transfer of processes for the manufacture of biocatalysts, small molecule APIs, and biologics. He earnt his PhD in biochemistry from Queen's University Belfast, In his current commercial role, Andrew focuses on the Charles River plasmid DNA manufacturing platform, eXpDNA™, and plasmid CDMO service offering.

AMANDA WEISS is the VP of CMC at Purespring Therapeutics, a kidney gene therapy company. Prior to this she was responsible for Validation & Commercialization for the lead ophthalmology product, Choroideremia, at Nightstar, a retinal disease gene therapy company, and also at Biogen post Nightstar's acquisition. She has over 27 years of industrial experience in the development, manufacture, and analysis of biological products and has held posts at both product development companies and CDMOs. She trained as a Biochemical Engineer at the University of Birmingham.

AFFILIATIONS

Andrew Frazer
Associate Director, Scientific Solutions,
Charles River Laboratories
Amanda Weiss
Vice President CMC,
Purespring Therapeutics

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

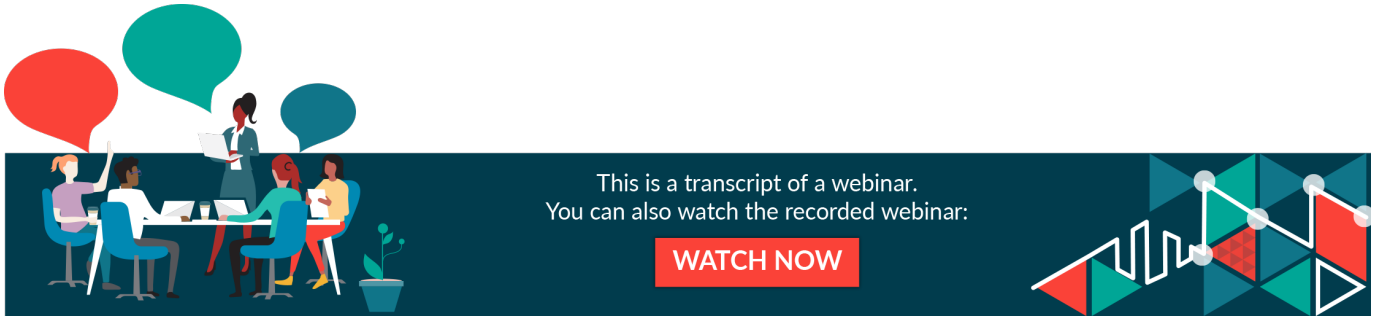
Disclosure and potential conflicts of interest: The authors have no conflicts of interest.

Funding declaration: The authors received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: This article is a transcript of a webinar, which can be found here.

Webinar recorded: Jan 24 2023; **Revised manuscript received:** Apr 19 2023; **Publication date:** May 3 2023



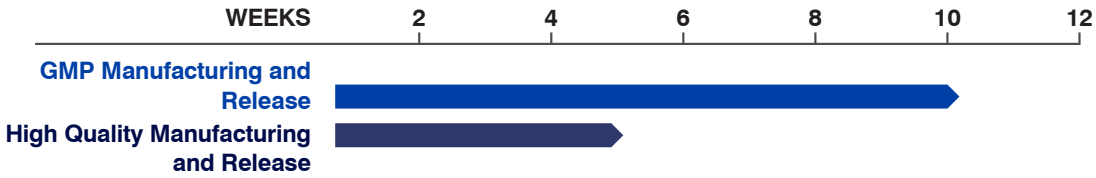
charles river

Cell and Gene Therapy, CDMO

eXpDNA™ Plasmid Development and Manufacturing Platform

Expediting pDNA Production and Supply

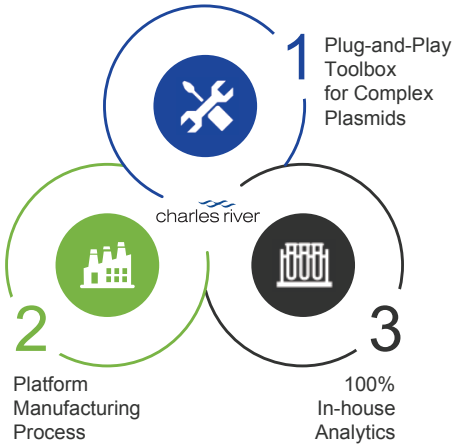
Focused on product quality, eXpDNA™ supports your plasmid strategy by offering a universal, standardized platform to streamline the development pathway and expedite production timelines to as little as five weeks.



eXpDNA™ Platform At-A-Glance

Fine-tuned by successfully supporting vaccine and advanced therapy medicinal product (ATMP) clients in the development, manufacture, and release of more than **200 High Quality (HQ) and GMP plasmid DNA batches to date**, eXpDNA employs a robust a plug-and-play screening toolbox for tackling challenging plasmids, fit-for-purpose production facilities, on-hand materials, and in-house analytics.

This combination, in addition to significant capacity expansion, enables the expedition of batch turnaround times to as few as **five weeks for HQ and ten weeks for GMP grade**.



CELL & GENE THERAPY INSIGHTS

VECTOR BIOPROCESSING & RAW MATERIALS

SPOTLIGHT

Transfection optimization for AAV production

Peter Boyce
Director, Process Development, AviadoBio

"...an optimized transfection process is essential to ensure optimal yields, quality, and consistency."

VIEWPOINT

Cell & Gene Therapy Insights 2023; 9(3), 305–309

DOI: 10.18609/cgti.2023.048

PRODUCTION OF rAAV IS A COMPLEX BIOLOGICAL PROCESS

Recombinant adeno-associated virus (rAAV) has emerged as the vector of choice for *in vivo* gene therapies. These vectors can be used to transduce mammalian cells, resulting in stable episomal maintenance and transgene expression. Recombinant AAV is

a replication-incompetent viral vector which is produced by one of several methods: baculovirus transduction of insect cells, plasmid transfection of mammalian cells along with a helper virus such as herpes simplex virus, or triple transient transfection of mammalian cells. More recently, stable producer cell lines have become more prominent, such as the ELEVECTA platform from CEVEC/Cytiva, due to the scalability of these platforms and reducing required amounts of costly raw materials such as plasmid DNA. Alternatives to plasmid DNA also exist: 'doggy-bone' DNA (dbDNA) is a synthetic DNA vector produced using an enzymatic DNA manufacturing process, completely removing the need for microbial culture.

Triple transient transfection of suspension-adapted HEK293 cells using plasmid DNA is one of the most common methods for production of AAV. This process does not rely on a helper virus, with the necessary helper elements being provided by plasmid (pHelper). Gene of interest is on a separate plasmid between two inverse terminal repeat (ITR) sequences (pGOI), and a third plasmid providing the replication and capsid proteins (pRepCap) [1]. The HEK293 cell line is commonly used due to the ease of culture and transfection, it is also a well characterized cell line, having been used for production of recombinant proteins since immortalization of the cell line in 1977. HEK293 cells also stably express adenovirus E1A and E1B, both of which are required for AAV replication [2].

Plasmid delivery into cells is assisted by a transfection reagent such as polyethyleneimine (PEI). PEI forms complexes with the plasmid DNA, and during incubation with the cells, binds to the cell membrane through electrostatic interactions. These complexes are then internalized via endocytosis in the form of an endosome. The endosome must traffic through the cytoskeletal network by way of microtubule interactions, avoiding lysosomal degradation until the plasmid DNA escapes from the endosome into the cytoplasm, and must then enter the nucleus through a nuclear pore complex (NPC) [3]. DNA entry into the nucleus is dependent on cell cycle and it has been suggested that transfection close to M-phase would be ideal, as the nuclear membrane breakdown can increase plasmid entry into the nucleus [4,5].

Optimization efforts with triple transient transfection have resulted in increased titers post-harvest, though for production of drug for systemic dosing, the current titers are still not sufficient as doses exceeding 10^{15}vg/patient may be required [6]. The simplest approach to this involves scaling up to massive production volumes in order to meet industry demand, but this has a huge effect on cost-of-goods for what are already expensive therapies. There are steps that can be taken prior to transfection optimization to optimize the production process, the most fundamental of which is selection and development of a suitable cell line. Cell line selection will dictate the upstream process in terms of adherent or suspension-cultured cells. Once selected, further development can take place such as clonal selection for a high-performing clone. Cell line engineering can also be utilized to knock-in or knockout specific genes to increase cell line performance. Plasmid engineering can also be carried out at this point to increase the proportion of full capsids produced. Potential targets for plasmid engineering could include modifications to the ITRs to increase full gene of interest (GOI) encapsidation, or modifications to the Rep protein to attempt to increase genome replication and packaging of the GOI [7].

One key challenge for HEK293-based systems is to ensure proper transfection. It has been reported that only 5–10% of cells appear to produce measurable levels of AAV capsid, suggesting that ensuring triple transfection of each plasmid into each cell may be an obstacle to overcome [8]. This could also be caused by the induction of antiviral and inflammatory responses, which have been reported to be a response to rAAV production in host cells [9], influencing these pathways by reducing or removing the ability of cells

DOI: 10.18609/cgti.2023.048

to respond to viral production may also provide an opportunity to increase viral titers. The addition of small molecule inhibitors to the cell culture can achieve this, though questions may be raised on the subsequent removal of these small molecules during downstream processing (DSP), or through cell line engineering to knockout or knockdown the relevant pathways.

The transfection process becomes more challenging as scale increases, which is becoming increasingly relevant as viral vector manufacturing facilities are more and more frequently designed for scales of more than or equal to 2000 L.

DoE VERSUS OFAT APPROACH

A critical step in optimizing transfection is identification of key factors in the process. These vary from raw materials such as transfection reagent, plasmids, cell line, and complexation media, through to factors such as complexation time, plasmid ratios, and total amount of DNA used. At larger scales, additional factors come into play such as time taken for, and efficiency of, dispersion of transfection mix within the reactor vessel. These factors may not be considered during initial process development activities, which typically take place at much smaller volumes. They can also be affected by variables such as reactor shape, volume, and impeller shape and speed.

As stated above, there are numerous factors affecting the efficiency of transfection for rAAV production. A common process for optimization is modification of one factor at a time (OFAT) – however, this does not account for interactions between the factors being investigated. Zhao *et al.* reported that an OFAT approach improved production of rAAV of a single serotype, but this increase was not observed when producing other serotypes [10].

Design of experiments (DoE) is an approach that has been successfully utilized in optimization of processes throughout the biotechnology industry. DoE allows the evaluation of multiple interdependent factors on a specified output. The first reported use of DoE to optimize AAV vector production was Zhao *et al.* in 2020, where the plasmid ratios, total DNA concentration, and cell density were simultaneously varied across 52 different conditions, resulting in average post-purification yields of more than 1×10^{14}vg/L across 13 different capsid variants [10]. Outcomes such as this can be used as an excellent baseline for new programs or products entering the pipeline, so reduced process development may be acceptable in these cases, without one needing to repeat the full DoE process from first principles.

Using DoE to optimize transfection will typically involve several rounds of experiments. The first round may look at a larger range of several variables, such as viable cell density at transfection, DNA concentration, transfection reagent:DNA ratio, transfection volume, and incubation time for complex formation. Following this, another DoE may be carried out to further refine these factors, or new variables may be brought in such as plasmid ratio. It would be necessary to repeat these DoE when introducing a new raw material (e.g. a new culture media), evaluating a new transfection reagent, or using a new cell line.

In recent years, more resources have been allocated to understanding the fundamentals of AAV production, both wild-type and recombinant. This has resulted in publications reviewing the proteomic landscape of AAV-producing HEK293 cells, reviews collating information to summarize the cellular pathways and kinetics of AAV production, and the development of mechanistic models for production of rAAV via triple transfection of HEK293 cells [11–13]. This deeper understanding of the cellular processes and kinetics allows for more targeted process optimization. An example of this would be utilizing a mechanistic model for PEI-mediated delivery of plasmid DNA, which has demonstrated that only 5% of total plasmid

input enters the cells, and the amount trafficked into the nucleus is even smaller at approximately 0.6% [13]. These figures may vary depending on the cell line and the transfection reagent, but do provide insight into the obstacles to be overcome when attempting to optimize the most critical part of the transient expression process.

OPTIMIZED TRANSFECTION IS A KEY STEP, BUT NOT THE ONLY STEP

When using a plasmid-based transient expression system, an optimized transfection process is essential to ensure optimal yields, quality, and consistency. The process of delivering all necessary plasmid DNA to the nucleus of the cells is fundamental to the successful development of a high-yielding production platform.

There are other factors which will need to be assessed to maximize productivity. These range from cell line development, omic assessment of the cell line to determine targets for knockout or knock-down of genes, and plasmid engineering to maximize full capsids, to media constituents and feeding regimes during production to optimize cell health and productivity. All of these factors and many others contribute to a production process which is sufficient to meet the needs of the current generation of gene therapies in development.

BIOGRAPHY

PETER BOYCE is Director, Head of Process Development at AviadoBio. He has 20 years' experience in the pharmaceutical industry , working in large pharma and small biotech in both GMP and non-GMP environments. His main areas of expertise are in biopharmaceuticals, such as mAbs and mAb-like molecules, and ATMP's. He has a BSc and MSc in Biomedical Sciences.

REFERENCES

1. Xiao X, Li J, Samulski RJ Production of high-titer recombinant adeno- associated virus vectors in the absence of helper adenovirus. *J. Virol.* 1998; 72, 2224–2232.
2. Graham FL, Smiley J, Russell WC, Nairn R. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J. Gen. Virol.* 1977; 36, 59–74.
3. Lechardeur D, Sohn KJ, Haardt M *et al.* Metabolic instability of plasmid DNA in the cytosol: a potential barrier to gene transfer. *Gene Ther.* 1999; 6, 482–497.
4. Schwake G, Youssef S, Kuhr JT *et al.* Predictive modeling of non-viral gene transfer. *Biotechnol. Bioeng.* 2010; 105, 805–813.
5. Brunner S, Sauer T, Carotta S, Cotten M, Saltik M, Wagner E. Cell cycle dependence of gene transfer by lipoplex, polyplex and recombinant adenovirus. *Gene Ther.* 2000; 7, 401–407.
6. Penaud-Budloo M, François A, Clément N, Ayuso, E. Pharmacology of recombinant adeno-associated virus production. *Mol. Ther. Methods Clin. Dev.* 2018; 8, 166–180.
7. McCarty DM, Fu H, Monahan PE, Toulson CE, Naik P, Samulski RJ. Adeno-associated virus terminal repeat (TR) mutant generates self-complementary vectors to overcome the rate-limiting step to transduction *in vivo*. *Gene Ther.* 2003; 10, 2112–2118.
8. Dash S, Sharon D, Mullick A, Kamen A. Only a small fraction of cells produce assembled capsids during transfection-based manufacturing of adeno-associated virus vectors. *Biotechnol Bioeng.* 2022; 119, 1685–1690.
9. Chung C, Murphy C, Wingate V *et al.* Production of rAAV by plasmid transfection induces antiviral and inflammatory responses in suspension HEK293 cells. *Mol. Ther. Methods Clin. Dev.* 2023; 28, 272–283
10. Zhao H, Lee KJ, Daris M *et al.* Creation of a High-Yield AAV Vector Production Platform in Suspension Cells Using a Design-of-Experiment Approach. *Mol. Ther. Methods Clin. Dev.* 2020; 18, 312–320.

DOI: 10.18609/cgti.2023.048

11. Strasser L, Boi S, Guapo F *et al.* Landscape of Adeno-Associated Virus(AAV)-Producing HEK293 Cells. *Int. J. Mol. Sci.* 2021; 22, 11499.

12. Sha S, Maolney A, Katsikis G *et al.* Cellular Pathways of recombinant adeno-associated virus production for gene therapy. *Biotechnology Adv.* 2021; 49, 107764.

13. Nguyen T, Sha S, Sun Hong M *et al.* Mechanistic model for production of recombinant adeno-associated virus via triple transfection of HEK293 cells. *Mol. Ther. Methods. Clin. Dev.* 2021; 21, 642–655.

AFFILIATION

Peter Boyce
Director,
Process Development,
AviadoBio

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given his approval for this version to be published.
Acknowledgements: None.
Disclosures and potential conflicts of interest: The author has no conflicts of interest.
Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited.
Revised manuscript received: Mar 30 2023; **Publication date:** Apr 3 2023

CELL & GENE THERAPY INSIGHTS

VECTOR BIOPROCESSING & MATERIALS

SPOTLIGHT

INNOVATOR INSIGHT

Determination of physical viral vector titer in process development & QC for cell & gene therapy

Daniel Forsström & John Chappell

The rapid and efficient development, production, and release of viral vectors for cell and gene therapy depends on high-performance analytics that support process understanding and enable QC for release testing. Critical quality attributes (CQAs) include total physical viral vector titer and the ratio of full to empty capsids, which are the focus of this article. A range of analytical methods to measure these CQAs are being evaluated to support process analytical technology (PAT) and QC. While advanced methods such as analytical ultracentrifugation (AUC) and cryo-electron microscopy (cryo-EM) are in use or being evaluated to analyze the highly pure final product, the analysis of multiple samples of lower purity during process development requires another approach. A common method to determine the full:empty capsid ratio during process development involves combining genome data from quantitative PCR (qPCR) or, more recently, droplet digital PCR (ddPCR), with capsid titer data determined by enzyme-linked immunosorbent assay (ELISA). While ddPCR is an effective and precise assay method, ELISA has several drawbacks, including low throughput, high sample consumption, labor-intensive steps, and long turnaround times. The generation of viral vector capsid titer can be streamlined by using the Gyrolab system, which enables the miniaturization and automation of immunoassays to address these drawbacks.

Cell & Gene Therapy Insights 2022; 9(3), 399–411

DOI: 10.18609/cgti.2023.060

INTRODUCTION

Since the first clinical trial conducted in 1990, major advances have been made in gene therapy, including the development of much-improved vectors. To date, most gene therapies utilize viral vectors, mainly adeno associated virus (AAV) and lentiviral (LV) vectors, to deliver the gene of interest. The major difference between LVs and AAVs is genome integration. LVs integrate their DNA into the host genome. This, together with the ability to express multiple genes means that LV vectors are frequently used to treat complex disease states such as congenital diseases, immune and metabolic disorders, and cancers. Genomic integration by LV prevents the dilution of genetic material over time due to cell division but poses a risk of oncogenesis. This problem is being addressed by third-generation, self-inactivating LV vectors that reduce the risk of insertional mutagenesis.

In contrast, genes delivered by AAVs become an episome, or circular piece of DNA that resides inside the nucleus. While a genome of ~4,7 kilobases (kb) limits the ability of AAV vectors to effectively package much more than ~5 kb, their extensive viral tropism means that AAV vectors are valuable for targeting gene therapies involving the heart, liver, and central nervous system.

Gene therapies represent over half of the 3726 gene, cell, and RNA therapies currently in development, with cancer and rare diseases as the main targets [1]. The revolution in gene therapy development is putting significant pressure on bioprocess development and quality control (QC) to ensure that vectors can be quickly brought to the market.

This article focuses on one aspect of ensuring vector safety and efficacy – the determination of physical titer including empty, full, and partially-filled capsids, in process development and QC. We start by briefly looking at the regulatory landscape concerning vector analytics, particularly physical titer, and then summarize how different analytical methods fit the needs for determining titer in viral vector process development, production, and release testing. We conclude by illustrating how the measurement of capsid titer can be refined to ensure high data quality and productivity with an example involving the miniaturization and automation of immunoassays.

THE REGULATORY LANDSCAPE

The US Food and Drug Administration (FDA) published revised regulatory guidelines for cell and gene therapy in January 2020 [2]. Overall, the FDA requires more detailed characterization and regulatory documentation for viral vector analytics regarding impurities, replication, titer, and infectivity.

The critical quality attributes (CQA) as mandated by the FDA's chemistry, manufacturing, and control (CMC) guidelines for viral vector manufacturing include identity, strength/potency, purity, safety, and stability to ensure safety and efficacy. More specifically, the CQAs that should be monitored during development include "dosing units, genotypic or phenotypic variation, particle number and size, aggregation state, infectivity, specific activity (ratio of infectious to non-infectious particles or full to empty particles), biological activity or potency, and/or immunological activity)".

Regarding product-related impurities, the Guidance states that, "For viral vectors, typical product-related impurities may include defective interfering particles, non-infectious particles, empty capsid particles, or replicating recombinant virus contaminants. These impurities should be measured and may be reported as a ratio, for example, full:empty particles or virus particles:infectious units".

Similarly, the EMA guidelines on gene therapy products [13] state that, "The quantity of the drug substance should be established. For viral vectors, infectious titer should be quantified; the number of particles (infectious/non-infectious, empty/genome-containing) should also be determined.

DOI: 10.18609/cgti.2023.060

Particle to infectivity ratio should be included to define the content of the drug substance. For plasmids and other forms of nucleic acids, the quantity or concentration of nucleic acid should be established".

Specific release criteria have also been indicated. For example, the proposed draft guidance for FDA consideration on testing AAV products for empty capsids [4] states that, "it is recommended that a maximum release criterion of ≤ 30% consisting of empty capsids be established for drug product. Accordingly, more than or equal to 70% of the product should consist of primarily full genome capsids". However, Biophorum indicates that, "it is premature and impractical to set a minimal specification of less than 30% empty capsids to apply to all AAV-based gene therapies in development". This organization suggests that the industry continues with a QbD risk-based strategy toward setting capsid specifications depending on the product [5].

OVERVIEW OF TITER ANALYTICS

The prospect of ensuring that a virus vector has the required purity for safe and effective use can be daunting. The key is to adopt a risk-based testing strategy in process development and final QC that minimizes the testing effort needed while meeting regulatory requirements and ensuring patient safety.

Process analytical technology (PAT) within the framework of Quality by Design (QbD) is becoming more widely applied to viral vector manufacturing. This has stimulated the development of advanced analytics that can quickly provide reliable data, including in-line testing, to improve process understanding to boost yields, improve vector safety, and lower costs. Rapid data generation for CQAs such as physical titer, including the full:empty ratio, is essential if techniques such as Design of Experiment (DoE) are to be effective. Analytical methods must therefore be rapid, accurate, and robust. Another important aspect is the ability to generate substantial amounts of data from limited amounts of precious sample, especially since regulatory demands are increasing the number of tests needed for batch release.

The testing strategy for viral vectors should include analytical methods with several important attributes that ensure the rapid and efficient generation of reliable data.

The need for speed to treat a select few

Cell and gene therapy can offer spectacular successes, including the treatment of patients suffering from genetic disorders previously thought to be incurable. Some therapies have been developed and approved for a relatively broad population, for example in the treatment of hemophilia A and B, and beta-thalassemia. Other therapies are only developed for a select few suffering from rare diseases and place particularly high demands on drug development. Patient populations are small, with personalized medicine sometimes being refined to truly individualized medicine, making treatments very expensive. Added to that, treatments are often fast-tracked from phase I for accelerated approval. Long assay times also add to the bottleneck in the development, production, and final QC of new products [6,7], emphasizing the need for faster analytical approaches to characterize the therapeutic with regard to quality and titer [8].

The demand for rapid turnaround times means that there is little time to validate new bioanalytical assay technologies, and rapid assay development and sample analysis are critical factors in reducing development times.

Increasing demands on data quality

As the number of clinical studies for AAV and LV-based gene therapies grows, the regulatory authorities are emphasizing the importance of vector titer assay reproducibility and the measurement of full:empty capsid

ratios to facilitate dose comparison between clinical programs. For example, a recent workshop formulated a target of less than or equal to 15% precision for measurement of empty AAV capsids for early phase studies, which may require improvements in the reliability of analytical methods for viral vector titer [9,10].

Getting more data from smaller sample volumes

Viral vector production is an expensive process that produces very little final product. For example, the product of a 200 L bioreactor can be concentrated down to 20 mL. Added to that, regulatory demands have increased the number of analyses required for characterization, putting an even higher premium on analytical techniques requiring less sample. It was estimated by one chemistry, manufacturing and control (CMC) specialist that almost half of the viral vector production batch may be consumed during QC bioanalysis steps [9], which means that analytical methods that can process very small sample volumes are at a premium.

THE NEED TO MEASURE CAPSID IMPURITIES

Capsid content characterization is a major challenge that puts a lot of pressure on analytics. Inefficiencies in viral vector production result in a fraction of viral particles that fail to package the vector DNA properly. This results in impurities that include empty capsids and capsids that contain nucleic acid sequences other than the desired vector genome.

TABLE 1

Distribution of AAV capsids during production.

Capsid type	Harvest (%)	Purified (%)
Full	< 30	>70
Partially filled	< 10	< 1
Empty	> 70	< 30

Estimates of the distribution of AAV capsids during production are summarized in Table 1. Data from [11]:

Aggregates can also be present at different levels (small less than 2% and large less than 1 ppm).

Taking AAV as an example, empty capsids can have several negative effects that threaten safety and efficacy [13]:

- Increasing the overall antigenic load that may exacerbate innate and adaptive immune responses;
- Contributing to the peptides presented by major histocompatibility complex (MHC) molecules, with consequent recognition and clearance of transduced cells by capsid-specific cytotoxic T cells;
- Functioning as a pathogen-associated molecular pattern (PAMP) that can be recognized by toll-like receptor (TLR) 2, resulting in the induction of innate immune responses;
- Competing with full capsids for receptor binding, which could necessitate a dose increase.

While the presence of empty capsids can have benefits in certain situations, for example as decoys for anti-AAV antibodies to enhance gene transfer, minimizing the level of empty capsids generally improves safety, especially when high vector doses are administered in clinical studies [13]. Removing AAV empty capsids during manufacture is a real challenge, especially during scale-up, which means that reducing the load by optimizing upstream and downstream processes is critical.

A recent draft guidance regarding AAV testing for FDA consideration [6] proposed identifying the following product impurities:

- Empty capsids;
- Non-infectious AAV;

DOI: 10.18609/cgti.2023.060

- Aggregated AAV;
- Replication-competent AAV;
- Encapsidated host-cell DNA;
- Encapsidated helper plasmid DNA;
- Encapsidated partial genome*;
- Encapsidated mutated* or methylated genome;
- Capsid post-translational modifications (PTMs)*.

* not included in prior FDA recommendations

ANALYTICS FOR CAPSID TITER CQAS

Several reviews have summarized the wide range of analytical methods available for determining capsid titer and genome titer [14–17]. In a draft guidance for FDA consideration, the consulting firm Dark Horse narrowed the field by proposing the methods shown in Table 2.

There are additional aspects of these techniques that should be pointed out:

- Charge detection mass spectrometry (CDMS) measures the charge and mass-to-charge ratio of individual ions and can be used to resolve empty, partially filled, and full capsids with a repeatability of less than 2% CV and a turnaround time of 2 h. But the method is less mature than, for example, analytical ultracentrifugation (AUC) [11].
- Transmission electron microscopy (TEM) has shown problems with poor agreement with orthogonal methods, low throughput, and long turnaround time [11].
- AUC is highly repeatable (2% CV) and can be used to resolve partially filled, empty, and full capsids. But this method consumes a lot of material (400–500 µL sample) and has a throughput of only seven samples in 6 h, making it more suitable as an orthogonal method to validate more rapid methods [11].

TABLE 2

Analytical methods for determining capsid titer and genome titer, as proposed by Dark Horse.

Method	Throughput	Ease of use	Material used	Partial genomes	Accuracy/ precision
Charge detection mass spectrometry (CDMS)	+ But requires buffer exchange	- Specialized equipment	++	++	++
ELISA + ddPCR	+ But d(d)PCR requires sample treatment	++ Commonly used	++	-	-
Size exclusion chromatography with multi-angle light scattering (SEC-MALS)	++	+ Relatively common equipment	+	-	+
Transmission electron microscopy (TEM)	- Sample staining, low throughput	- Specialized equipment	++	-	-
Analytical ultracentrifugation (AUC)	-	- Specialized equipment	+	++	++

Based on guidance proposal from Dark Horse [4].

- Methods to measure partially filled capsids include AUC and CDMS shown in Table 2, and also Cryo-EM [12].

A common approach used today for measuring the full:empty ratio therefore involves measuring the genome content and capsid content separately and then using the quotient to determine the % of full:empty capsids. Quantitative polymerase chain reaction (qPCR) or digital droplet PCR (ddPCR), which is replacing qPCR, are widely used methods to quantify genome titer due to their simplicity, specificity, and robustness. They are based on fluorescence detection of specific DNA sequences during amplification (qPCR) or after amplification (ddPCR) in a thermocycler. Both require sample preparation to remove non-encapsidated DNA and denature capsid proteins to expose the encapsidated DNA. qPCR is the standard procedure for determining genome titer of rAAV reference standard material (RSM) but suffers from low precision, with repeatability as low as >30 %CV and reproducibility of 70–100%. In contrast, ddPCR, which does not require a standard curve and measures the endpoint of PCR cycles, has a repeatability of 2–20 %CV [11].

ELISA is the most common method for determining capsid titer and has a high specificity for intact capsids and is relatively robust to matrix effects. This method can deliver acceptable performance when used to determine AAV capsid titer, with a repeatability of 10–15 %CV and reproducibility of around 40 %CV [11]. This traditional plate-based method suffers from several disadvantages, however, including low throughput (10 samples per 96-well plate), high sample consumption, and requires labor-intensive steps together with turnaround times of several hours.

TRANSLATIONAL INSIGHT

Miniaturization & automation boost immunoassay performance

The combination of data from qPCR or ddPCR and ELISA is often used to generate data on full:empty capsid ratios, but data quality can be compromised by the accumulated error resulting from combining results from

TABLE 3

The key factors in choosing an immunoassay platform.

Key factors	Benefit
High precision and accuracy	Confidence in decisions
Broad analytical range	Reduces need for dilutions and repeats
Robustness	Reliable and repeatable data
Matrix tolerance	Enable the analysis of complex samples with low minimum required dilution (MRD), which improves functional sensitivity.
Rapid data generation	Meet tough timelines
High throughput	Efficiently handle large sample sizes in development
Flexible open platform	Run multiple assays in parallel to save time Enable the development of novel assays
Automation	Free up scientist's time for other critical tasks Reduces risk of error
Low sample- and reagent consumption	Ensure maximum data generation with the minimum of precious samples and reagents
Easily sanitized	Meet biosafety requirements when working with viral vectors
Readily validated and 21 CFR Part 11 compliant software	Meet the demands of regulatory guidelines

DOI: 10.18609/cgti.2023.060

two analytical methods. The shift from qPCR to ddPCR can improve the repeatability of genome measurements but there remains a need for the efficient and rapid determination of physical capsid titer with high accuracy and repeatability. ELISA is a well-established method to determine capsid titer but has relatively low throughput, narrow analytical range, requires many manual steps, and consumes relatively large volumes of sample. The question is, how the immunoassay-based determination of capsid titer can be improved to support the rapid generation of high-quality data?

The key factors in choosing an immunoassay platform are summarized in Table 3.

Gyrolab system has been developed by Gyros Protein Technologies to address the requirements listed in Table 3 and is now well established in the biotech and pharmaceutical industry for a wide range of applications, including vector quantitation and characterization, host cell protein impurity measurement, and monitoring *in vitro* potency. Kits are available to determine titers of AAV serotypes 1–10 and the p24 antigen of LV. The principle of a Gyrolab assay is shown in Figure 1.

The automation and miniaturization of the flow-through assays afforded by Gyrolab technology results in several benefits over plate-based ELISA (Table 4 & Figure 2).

When compared to ELISA kits, Gyrolab microfluidic immunoassays greatly reduce the sample volumes, hands-on time required, and overall assay time, while extending the assay dynamic range. These dramatic improvements in assay performance and sample consumption meet the demands for vector titer bioanalysis required by the compressed production timelines and limitations on batch yields.

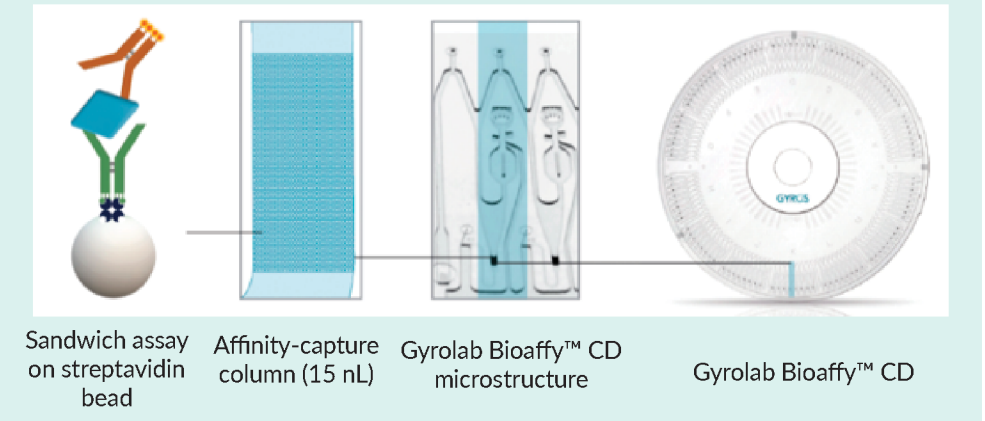
The high quality of data generated using Gyrolab assays can be seen in Figures 2–4 and Tables 5 & 6.

In the context of ICHQ2(R1) [18], these data summarize repeatability (intra-assay precision), intermediate precision (inter-run precision), linearity, and range.

Gyrolab AAVX Titer Kit has working ranges of 1×10^{8}–1×10^{11} for serotypes AAV1 – AAV7 and AAVrh10, and 1×10^{9}–1×10^{12} for

FIGURE 1

Gyrolab® Bioaffy™ CD-based microfluidic immunoassay design utilizing a 15 nL affinity capture column, streptavidin beads, and microstructures in a circular array for precise, automated liquid movements using centrifugal force.



Parallel processing of Gyrolab CD-based immunoassays on streptavidin beads within the affinity capture column uses centrifugal force and capillary action to precisely control the flow of reagents and samples over the column. On-column laser-induced fluorescence results are read automatically, and results are ready to analyze at the end of the run. The short contact times minimize matrix interference and dramatically shorten assay times.

TABLE 4

Performance of Gyrolab AAVX capsid titer immunoassay exceeds ELISA performance and suitability for bioprocess development.

	ELISA	Gyrolab system
Sample volume required	100–200 µL	8 µL
Number of hands-on steps	5	1
Total assay time	4 h	1 h
Dynamic range	1–2 logs	> 3 logs

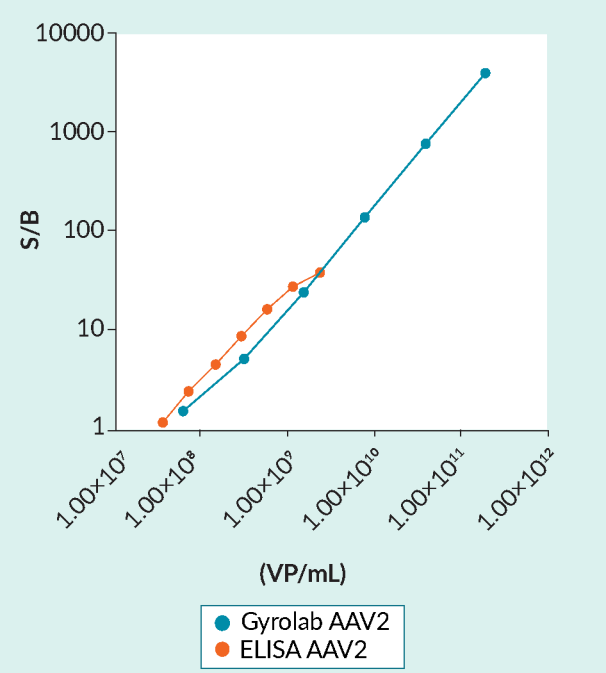
AAV8. A separate kit is available to measure AAV9 titer. Table 5 shows data for standard curves and QC samples over the working range of the Gyrolab AAVX Titer Kit when used to measure AAV2 titer. Samples were run in duplicate in six runs on four instruments by three operators. Six duplicate runs were performed on four different instruments, or N=12 per standard concentration. The intra- and inter-run precision was well under 10% (1.7–5.3%), demonstrating an extremely robust assay.

Table 6 shows similar accuracy and precision data for the quantification of LV capsid titer by determining p24 antigen using Gyrolab 24 Kit.

Further support for the suitability of Gyrolab system in the determination of capsid titer is shown in Figures 3 and 4. Gyrolab assays deliver comparable data to ELISA when used to analyze a range of samples from upstream and downstream processing (Figure 3) and the assays show high dilutional linearity (Figure 4).

FIGURE 2

Gyrolab AAVX capsid titer immunoassay performance versus ELISA.



The broad dynamic range of Gyrolab AAV immunoassays reduces the need to dilute or re-run samples. The 2-log increase in dynamic range is especially useful in high-titer AAV batch production. The Gyrolab AAV2 immunoassay was performed using Gyrolab AAVX Titer Kit. ELISA was performed according to the kit instructions (PROGEN). AAV2 standards (Sirion Biotech GmbH) were measured in duplicate after dilution in steps of 1:5 from 2.0×10^{11} VP/mL or in steps of 1:2 from 2.4×10^{9} (ELISA). (S/B, signal/background; VP/mL, viral particles per mL).

DISCUSSION

Rapid advances in cell and gene therapy include the development and evaluation of a wide range of analytical techniques to determine the CQAs needed to guide process development and support QC and final release testing. Advanced methods such as AUC and cryo-EM for final release testing are being evaluated to generate data on empty, full, and partially filled capsids in one analysis but require complex instrumentation. Immunoassays, on the other hand, are based on readily available instrumentation and can generate data relatively quickly to support process development in particular.

The determination of CQAs such as capsid titer, including full:empty ratios, for process development and final QC, relies on the availability of analytics that can quickly deliver high-quality reliable data with a minimum of effort and sample. Plate-based

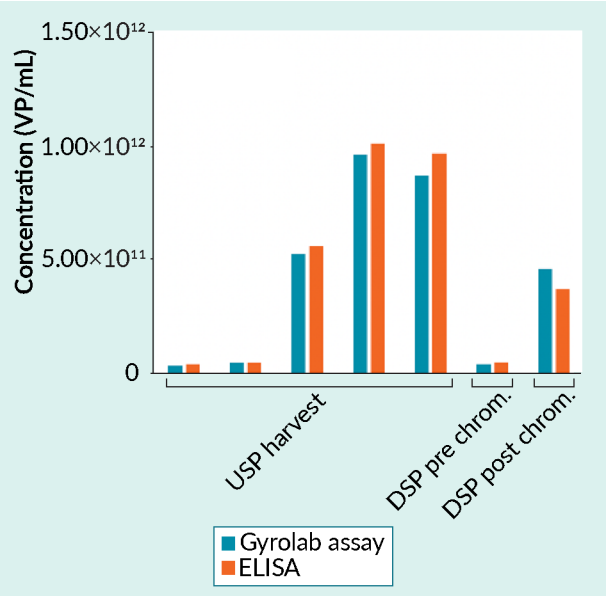
DOI: 10.18609/cgti.2023.060

immunoassays (ELISA) are commonly used to measure physical virus/capsid titer in process development and QC. Measuring the CQA, empty:full ratio, means combining the capsid data with genome data generated using PCR-based methods, which results in accumulated error and necessitates the development of individual methods with high precision. The need for high precision, for example, was noted in an interview with Christine Le Bec in *Cell & Gene Therapy Insights*, with a target for precision of less than or equal to 15% CV for the measurement of empty AAV capsids being recommended for early phase studies [9].

In the case of genome determinations, the need for increased precision has resulted in a shift from using qPCR to ddPCR to improve data quality. On the other hand, the generation of capsid data using plate-based immunoassays has several disadvantages, including the need for large sample volumes, relatively laborious and time-consuming workflows, and

FIGURE 3

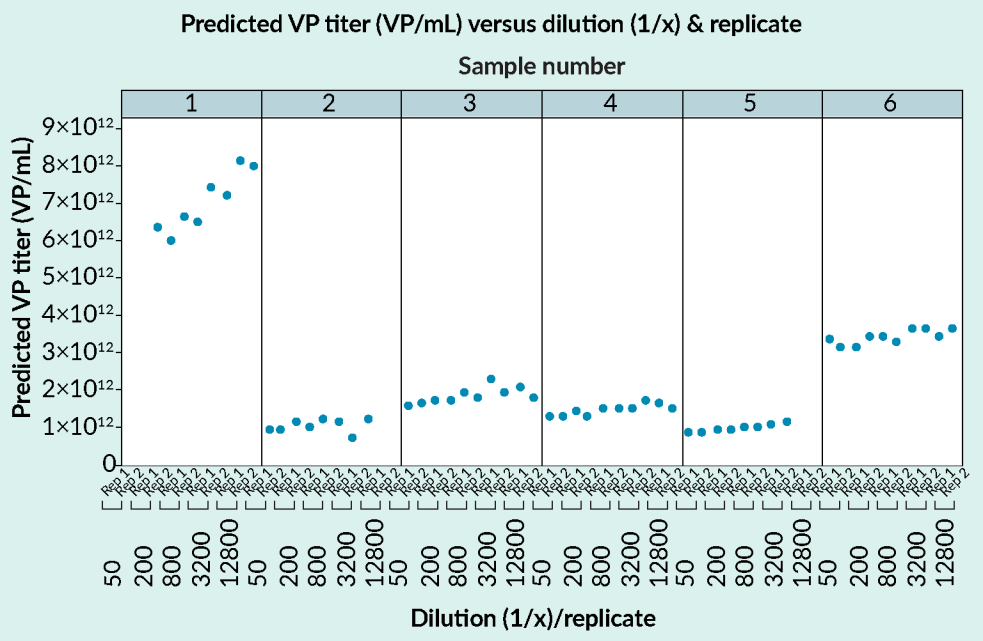
Analyzing samples from upstream and downstream processing: Gyrolab assay versus ELISA.



Gyrolab AAV9 Titer Kit and a manual capsid ELISA gave comparable results for a range of samples. The data was supplied by a CRO providing analytical services for cell and gene therapy customers. USP, upstream process; DSP, downstream process.

FIGURE 4

Gyrolab AAVX Titer Kit results in the determination of capsid titer for AAV5.



Gyrolab AAVX Titer Kit shows good dilutional linearity with comparable determinations for viral particle (VP) titer whether the sample was diluted 1:50 or 1:12800. Data is for serotype AAV5.

TABLE 5

Gyrolab AAVX Titer Kit: representative accuracy and precision data for seven QC samples for the determination of the working range when determining AAV2 capsid titer.

Sample	Expected conc (VP/mL)	Average measure conc. (VP/mL)	Intra-run CV (%)	Inter-run CV (%)	Average accuracy (%)	Average TE (%)
ULOQ 1	1.87×10^{11}	1.86×10^{11}	1.67	2.8	99.3	0.93–8.16
ULOQ 2	1.26×10^{11}	1.22×10^{11}	2.52	5.3	96.6	2.47–12.24
MQC	9.16×10^{9}	9.27×10^{9}	4.03	4.1	101.2	4.80–12.92
LQC	4.65×10^{8}	4.56×10^{8}	2.49	2.3	98.0	2.30–9.79
LLOQ 1	1.76×10^{8}	1.74×10^{8}	7.91	4.5	98.7	6.55–17.86
LLOQ 2	1.22×10^{8}	1.20×10^{8}	9.15	10.9	98.3	13.12–27.22
LLOQ 3	9.85×10^{7}	8.94×10^{7}	1.18	5.5	90.8	9.30–39.94

limited dynamic range. Gyrolab system has been developed to address these problems and illustrates how technology development can support cell and gene therapy in a similar way to the shift from qPCR to ddPCR for genome determinations. Gyrolab assays can quickly deliver data with a precision of better than 10 %CV, which matches the performance of ddPCR (repeatability 2%–10% CV [11]) to increase the precision of not only total capsid determinations but also full:empty ratios. The microfluidic design, flow-through affinity column, and automation all contribute to the high reproducibility both within runs and between runs.

Gyrolab system and associated kits can also be used to measure other impurities, such as host cell proteins (HCPs), endonuclease, and transferrin. The automation increases throughput and reduces risk of error, and the system is readily validated and is supported by 21 CFR Part 11 compliant software.

CONCLUSION

The application of Gyrolab technology represents just one example of the search for analytical methods with short turnaround times, high throughput, and simple sample preparation that deliver reliable data for a wide range of CQAs in vector development and production. These efforts will help address bottlenecks in vector production and support process understanding, with the goal of matching advances made in the production of other complex pharmaceuticals and the timely release of safe and efficacious cell and gene therapeutics.

TABLE 6

Gyrolab p24 Kit: Intra- and inter-run precision for the standards used to prepare the standard curve.

	Expected conc (ng/mL)	Average measured conc (ng/mL)	Intra-run CV (%)[1]	Inter-run CV (%)[2]
Blank				
Standard 1[3]	1250	1250	3.6	3.1
Standard 2	250	251	2.3	1.9
Standard	50	50	2.8	2.7
Standard 4	10	10	2.9	2.8
Standard 5	2	2	1.7	2.1
Standard 6	0.4	0.4	2.0	1.8
Standard 7	0.08	0.08	5.0	5.3

[1]Intra-run CV (%) = standard deviation of response divided by mean response from one run performed in duplicates.
[2]Inter-run CV (%) = standard deviation of means from six runs performed in duplicates divided by mean response for the six runs.
[3]Purified recombinant p24 standards diluted in assay buffer.

DOI: 10.18609/cgti.2023.060

BIOGRAPHIES

DANIEL FORSSTRÖM is R&D Application and Custom Service Manager at Gyros Protein Technologies. He leads the RnD Application and Custom service teams for Gyros Protein Technologies and is responsible for developing new assays, new products and custom assay solutions for the Gyrolab platform. He has obtained vast experience developing analytical assays for high throughput technologies used in preclinical and clinical drug development after approximately 15 years in various positions at different biotech companies.

JOHN CHAPPELL has approximately 25 years of experience in the Contract Research industry supporting both preclinical and clinical drug development. He has specialized in supporting biological compounds from an analytical perspective e.g. Pharmacokinetic, Immunogenicity and Biomarker analysis. He is particularly interested in validation requirements and ensuring that data generated will be acceptable to the regulatory authorities. He has spoken at many international conferences on various topics including Oligonucleotide analysis, Biomarker Analysis, Immunogenicity and the analytical support of Biosimilar programs. He now leads the Application Support and Service teams for Gyros Protein Technologies where he is responsible for customer service and technical support in Europe and the Asia Pacific regions. John has been a user of the Gyrolab® system for over 10 years so will use this experience to help customers. He is a Fellow of the Royal Society of Chemistry and was involved in the American Association of Pharmaceutical Scientists (AAPS) Biosimilar Committee that has prepared papers on Pharmacokinetic and anti-drug antibody assays.

AFFILIATIONS

Daniel Forsström
Gyros Protein Technologies

John Chappell
Gyros Protein Technologies

REFERENCES

1. American Society of Gene & Cell Therapy. Gene, Cell, & RNA Therapy Landscape report, Q4 2022.
2. Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs). US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research, January 2020.
3. European Medicines Agency. Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products. EMA/CAT/80183/2014. (March 22 2018).
4. Dark Horse Consulting. Proposed DRAFT Guidance for FDA Consideration: Testing of Adeno-Associated Viral (AAV) Vector-Based Human Gene Therapy Products for Empty Capsids During Product Manufacture. *Dark Horse Consulting Group*. (May 15 2022).
5. Biophorum. Control of empty full and partially filled capsids. Biophorum. (Accessed March 5 2023).
6. Rininger J, Fennell A, Schoukroun-Barnes L, Peterson C, Speidel J. Capacity analysis for viral vector manufacturing. Is there enough? BioProcess Int. 2019; 17(11).
7. Transfiguracion J, Tran MY, Lanthier S *et al.* Rapid in-process monitoring of lentiviral vector particles by high-performance liquid chromatography. *Mol. Ther. Meth. Clin. Dev*. 2020; 18, 803–810.
8. BioPharm. Gene Therapies Push Viral Vector Production – Bioprocess Development Forum. (May 21 2020).
9. Christine Le Bec. Priorities for AAV vector analytics development. *Cell Gene Ther. Insights* 2020; 6(7), 871–875.
10. US Food and Drug Administration. Quantitation of AAV-based gene therapy products – 12/07/2018. FDA.
11. Gimpel AL, Katsikis G, Sha S *et al.* Analytical methods for process and product characterization of recombinant adeno-associated virus-based gene therapies. *Mol. Ther. Meth. Clin. Dev*. 2021; 20, 740–754.
12. Subramanian S, Maurer AC, Bator CM *et al.* Filling Adeno-Associated

Virus Capsids: Estimating Success by Cryo-Electron Microscopy. *Hum. Gene Ther.* 2019; 30(12), 1449–1460.

13. US Food and Drug Administration. Toxicity Risks of Adeno-associated Virus (AAV) Vectors for Gene Therapy. Food and Drug Administration (FDA) & Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC) Meeting #70. September 2–3, 2021.

14. Hutanu A, Boelsterli D, Schmidli C, *et al*. Stronger together: Analytical techniques for recombinant adeno associated virus. *Electrophoresis.* 2022; 43(9–10), 1107–1117.

15. Shmidt AA, Egorova TV. PCR-Based Analytical Methods for Quantification and Quality Control of Recombinant Adeno-Associated Viral Vector Preparations. *Pharmaceuticals (Basel).* 2021; 15(1), 23.

16. Werle AK, Powers TW, Zobel JF *et al.* Comparison of analytical techniques to quantitate the capsid content of adeno-associated viral vectors. *Mol. Ther. Meth. Clin. Dev*. 2021; 23, 254–262.

17. Sylvestre J, Conti-Permanne P. Emerging technologies & companies in cell & gene therapy manufacturing. *Cell Gene Ther. Insights* 2022; 8(11), 1601–1649.

18. FDA Q2(R1) Validation of Analytical Procedures: Text and Methodology Guidance for Industry.

GYROS PROTEIN Technologies

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The authors are employees of Gyros Protein Technologies.

Funding declaration: The authors received financial support for the research, authorship and/or publication of this article from Gyros Protein Technologies.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited; externally peer reviewed.

Submitted for peer review: Mar 9 2023; **Revised manuscript received:** Apr 26 2023; **Publication date:** May 12 2023.

DOI: 10.18609/cgti.2023.060

Automate your immunoassays and compress development timelines

One-hour automated immunoassays in a nanoliter-scale offer low sample and reagent consumption and exceptional reproducibility to simplify and speed up lab work.

Learn how to automate immunoassays like ELISA with Gyrolab®

GYROS PROTEIN Technologies

Plasmid DNA Manufacturing and Analytics

The rapid increase of the gene therapy pipeline and genetic vaccination for various infectious diseases requires large-scale production of high-quality plasmid DNA (pDNA). In gene therapy applications, pDNA is a starting material for cell transfection. However, producing sufficient quantities of pDNA can be a challenge. Therefore, it is essential to establish robust manufacturing and analytics processes to produce large quantities of pDNA.

MARKET VALUE

Global plasmid DNA manufacturing market value 2022:
$400-700 million

APPLICATIONS OF pDNA

pDNA is used as a starting material in the manufacture of many advanced therapies. It can also be used as a therapeutic itself in DNA vaccines

- DNA vaccines & therapies, transient protein therapies
- Viral vector manufacturing
- Template for mRNA manufacture
- Gene-modified cell therapies (i.e., CAR-T cells)

CHALLENGES IN THE SUPPLY OF pDNA

- Complex supply chains
- Production and capacity bottlenecks caused by high demand
- Costs of goods
- Evolving regulatory landscape
- Quality assurance

MANUFACTURING CHALLENGES

- pDNA and contaminants are of similar size and charge
- Large size of product
- High negative charge
- pDNA is sensitive to shear stress and nuclease degradation
- Intermediate process pools can be highly viscous
- High volume of impurities in the starting material (<1% pDNA)
- Conventional chromatography resins exhibit low binding capacities for pDNA
- High purity is required

pDNA MANUFACTURE

PLASMID SYNTHESIS

- Sequence determination for gene of choice
- Gene creation
- Gene insertion into cloning vector

UPSTREAM

During the upstream process, pDNA is produced in host cells. *E. coli* is typically used as the host as they proliferate rapidly and therefore produce large quantities of pDNA.

Seed expansion & production

- Innoculation of the bioreactor using the seed train
- Fermentation (expansion of the cells, and resultant pDNA synthesis)

DOWNSTREAM

During the downstream process, pDNA is extracted from the host cells and purified from the host cell proteins, DNA and other impurities in the lysate

Cell harvest

Cells are concentrated and the fermentation broth is removed.

- Separation of cells can be achieved using centrifugation or microfiltration-tangential flow filtration.

Centrifugation is more cost-effective at particularly low or high volumes.

Shear damage due to centrifugation can reduce the yield of supercoiled plasmids

Extraction of pDNA from *E. coli*

Cells are lysed to release pDNA and cell debris and genomic DNA is removed.

- Chemical (alkaline) cell lysis with NaOH solution
- Neutralization with potassium acetate: precipitation of proteins, chromosomal DNA and some RNA

Clarification

At this stage, in addition to the pDNA, the precipitate will contain impurities, including host cell proteins and DNA. The clarification step removes cell debris and other impurities from the high-density cell culture

- Centrifugation: removal of precipitated proteins and chromosomal DNA
- Depth filtration: clarification of large particles

Concentration and diafiltration

Reduction of the sample volume and removal of smaller impurities.

- Buffer exchange
- Ultrafiltration/diafiltration using 100–300 kDa membrane (0.3 bar TMP)
- Sterile (0.2 µm) filtration

The large size and shear-stress sensitivty of the pDNA present a challenge at this stage.

Capture (chromatography)

Isolation of pDNA from contaminants. pDNA capture requires processes that are low shear and suitable for large molecules.

- Anion exchange chromatography (AEX)

Polish (chromatography)

Isolation of supercoiled pDNA from other isoforms (open circular and linear)

- Hydrophobic interaction chromatography (HIC)
  - Isolation of supercoiled pDNA

Supercoiled pDNA is the most stable, efficient, and biologically active form

Concentration and diafiltration

Concentration of the pDNA, reduction of sample volume, buffer exchange

- Diafiltration 100-300 kDa Membrane
- Buffer exchange into formulation buffer.

FILL AND FINISH

RELEASE TESTING

Concentration	Plasmid concentration
Characterization	Appearance
Identity	Osmolality, pH
Stability	Identity, Plasmid identity, Restriction digest
Purity	Purity by A260/280 ratio, DNA homogeneity, Plasmid form
Process residuals	Residual host cell DNA, Residual host cell protein, Residual host cell RNA, Detection of antibiotics (if applicable)
Safety	Endotoxin, Bioburden, Sterility (if applicable), Mycoplasma (if applicable), Container closure

ANALYTICS

pDNA is subject to stringent requirements for purity, efficacy, and yields.

There are three recognized plasmid quality grades, dependent on the intended use. The information in this infographic relates to the manufacture of GMP-grade plasmid.

ANALYTICS TESTING

Residual host cell protein:
- ELISA
- PCR

Host cell *E. coli* DNA:
- qPCR

Host *E. coli* RNA:
- Agarose gel electrophoresis (AGE)
- RT-qPCR,
- Fluorometric quantification

Identity:
- Restriction digest
- AGE

Nucleic acid concentration:
- Spectroscopy

Purity:
- Spectroscopy
- AGE

Supercoiled plasmid ratio:
- Analytical anion exchange chromatography
- Analytical hydrophogic interaction chromatography
- Analytical reversed phase chromatography
- AGE

Residual antibiotic:
- Immunoassay

Protein
- Colorimetric spectroscopy
- SDS-PAGE

Endotoxin:
- Kinetic chromogenic assay
- LPS assay

Sterility:
- Direct inoculation
- Membrane filtration sterility test

ThermoFisher SCIENTIFIC

CELL & GENE THERAPY INSIGHTS

VECTOR BIOPROCESSING & MATERIALS

SPOTLIGHT

Standardizing success: paving the way for streamlined viral vector manufacturing in gene therapy

Raquel Martín-Ibáñez
Associate Director for Process Development and Manufacturing
Stanford Center for Cancer Cell Therapy, Stanford Cancer Institute, Stanford University

"Continued research, development, and collaboration between academia, industry, and regulatory agencies will be key to advancing the field and ensuring that we are ready to build robust and scalable viral vector platforms."

VIEWPOINT

Cell & Gene Therapy Insights 2023; 9(3), 363–368

DOI: 10.18609/cgti.2023.056

The fields of gene therapy and cancer treatment have witnessed remarkable progress in recent years with the development of new gene therapies for genetic disorders and cancer. As these approaches show promising results in clinical trials and move toward regulatory approval and commercialization, there is a growing demand for Good Manufacturing Practice

(GMP)-grade viral vector manufacturing. However, several challenges must be addressed to optimize viral vector production, including selecting the right production system, optimizing downstream processing, and standardizing chemistry, manufacturing and control (CMC) methods and quality assays.

SELECTING THE RIGHT PRODUCTION SYSTEM

When selecting a production system, it is important to understand the specific requirements of the viral vector type, consider the desired production scale, ensure compatibility with the host cell line, evaluate the level of process control and automation, comply with regulatory requirements, consider cost and scalability, and assess process flexibility for optimization and customization. Depending on the application and the scale, several production systems may be available [1].

OPTIMIZING DOWNSTREAM PROCESSING

Several strategies can help improve the efficiency, cost–effectiveness, and product quality of viral vector production during downstream processing (DSP). These strategies include:

- Chromatography optimization is a key strategy in DSP. By carefully selecting and optimizing chromatography steps, such as resin selection, column packing, and elution conditions, viral vector purity and yield can be significantly improved. Fine-tuning filtration steps, including membrane selection, pore size, and filtration conditions, can also improve viral vector yield and quality. Process integration, which involves optimizing process flow and integrating DSP steps, can streamline the overall production process, reducing turnaround time and increasing productivity [2].
- Another critical aspect of DSP is analytical characterization. Real-time monitoring of critical quality attributes (CQAs) and process parameters using robust analytical methods allows for better control and characterization of the viral vector during DSP. This enables early identification and resolution of potential problems, ensuring the production of a high-quality product [3].
- Viral vector stability is of paramount importance during DSP [4]. Optimizing process conditions, such as temperature, pH, and agitation rates, helps maintain the stability and integrity of the viral vector during the purification process. Scale-up considerations are also critical, as parameters such as column size, flow rates, and residence times must be carefully optimized for smooth scale-up from small-scale to large-scale production.
- Finally, regulatory compliance cannot be overlooked. Adherence to regulatory guidelines is essential for ensure the safety, efficacy, and market approval of the viral vector product.

STANDARDIZING PROCESSES USING PROCESS OPTIMIZATION PRINCIPLES

Unlike monoclonal antibody manufacturing, viral-vector manufacturing is not standardized across the industry or academic institutions. This lack of standardization presents challenges in terms of cost, product quality, and regulatory compliance, particularly as gene therapies move toward commercialization and face increasing regulatory scrutiny. However, one approach that can help achieve standardization in viral vector production is through the application of process optimization principles [5,6]. By identifying and optimizing critical process parameters (CPPs) and CQAs, a robust and reproducible process can be developed,

DOI: 10.18609/cgti.2023.056

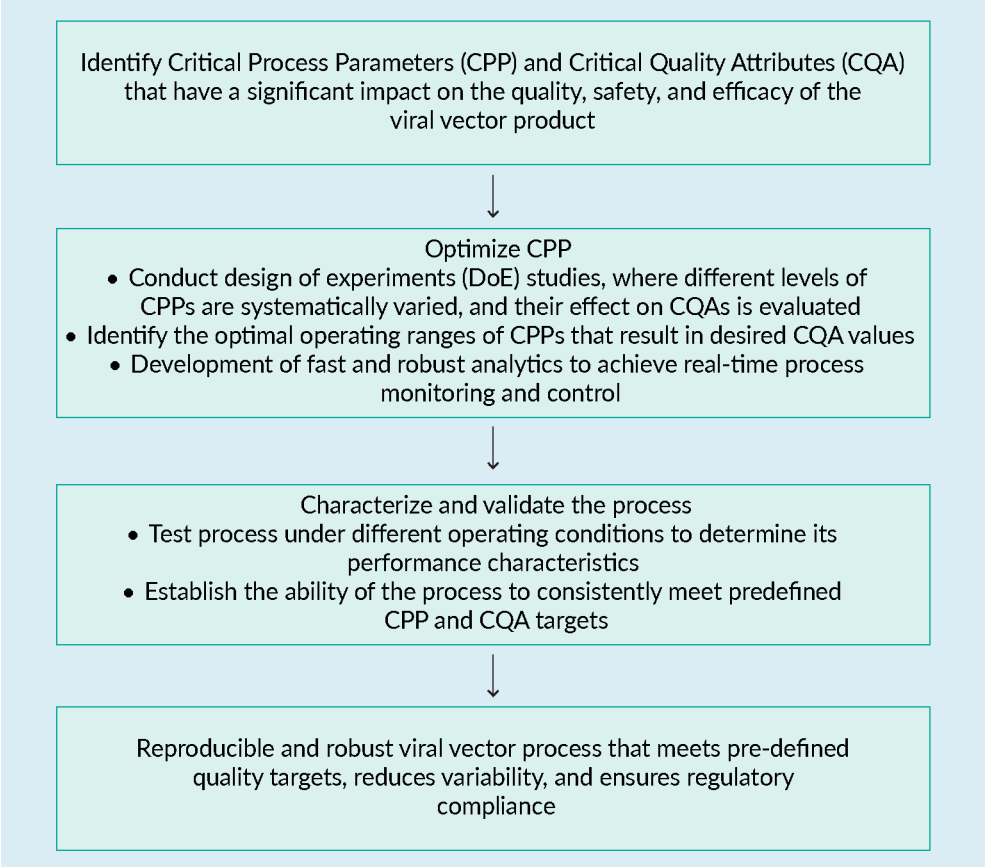
resulting in consistent product quality, reduced process variability, and improved cost–efficiency. Let's look at how process optimization principles can be applied to standardize viral vector production.

The first step in process optimization is to identify the CPPs and CQAs that have a significantly impact on the quality, safety, and efficacy of the viral vector product (Figure 1). CPPs are the process parameters that need to be carefully controlled and monitored to ensure process consistency and reproducibility. CQAs are the characteristics of the viral vector product that are critical in determining its quality, safety, and efficacy. Examples of CPPs in viral vector manufacturing include, but are not limited to, transfection or transduction methods, vector component concentrations, purification methods, and formulation buffer composition, among others. Examples of CQAs in viral vector manufacturing include, but are not limited to, vector yield, vector potency, vector purity, and vector stability, among others.

Once the CPPs and CQAs have been identified, the next step is to optimize the CPPs to achieve a robust and reproducible process. This may involve conducting design of experiments (DoE) studies, where different levels of CPPs are systematically varied, and their effect on CQAs is evaluated. Various statistical tools and design space analysis can be used to identify the optimal operating ranges of CPPs that result in desired CQA values. Optimization of CPPs may also involve the development of fast and robust analytics to achieve real-time process monitoring and control.

FIGURE 1

Process optimization steps to standardize viral vector production.



Once the optimal CPP ranges have been identified, the next step is to characterize and validate the process to ensure its reproducibility and robustness [5,6]. This may involve conducting process characterization studies, where the process is tested under different operating conditions to determine its performance characteristics. Process validation studies, including process capability studies, will help establish the ability of the process to consistently meet predefined CPP and CQA targets. Process characterization and validation studies are critical to demonstrating process control.

By implementing process optimization early in the development process, manufacturers can establish a reproducible and robust process that meets pre-defined quality targets, reduces variability, and ensures regulatory compliance (Figure 1). This is particularly important when transferring the process to a contract development and manufacturing organization (CDMO) or scaling up for commercial purposes.

BUILDING A PLATFORM

Recently, significant technological advances have paved the way for the development of robust viral vector platforms [1]. The success of existing viral vector platforms in producing high-quality vectors with improved timelines and reduced costs is a testament to the potential of the field. However, despite the significant progress that has been made, there are still areas that require further research, development, and optimization to unlock the full potential of viral vector manufacturing [7]. These areas include the development of novel bioreactor designs, which allow precise control of environmental conditions that can improve cell growth and vector production yields, as well as advanced transfection methods that are efficient, scalable, and cost–effective to streamline the manufacturing process. The use of animal-free media and reagents is gaining traction to reduce variability and the risk of contamination. Development of stable producer or packaging cell lines generated by gene editing technologies may improve scalability and consistency. Closed systems can minimize the risk of contamination and improve safety and reproducibility. Continuous or advanced downstream processing technologies improve the efficiency and yield of vector purification. Finally, there is a requirement for further development of new materials and single-use solutions for downstream processing.

Advancements in analytical assays are also critical to building a standardized viral vector platform [8]. Robust analytical methods are essential to determine the quality profile of the product, including safety, purity, potency, and stability, throughout the production system and in real time. Emerging technologies are being implemented such as next-generation sequencing (NGS) and mass spectrometry, rapid in-process testing methods such-us reverse transcription polymerase chain reaction (RT-PCR) or Droplet Digital PCR (ddPCR), process analytical technology (PAT) for real-time monitoring and control, advanced data analytics including machine learning and artificial intelligence (AI), automation and robotics, and emerging concepts like real-time release testing.

Finally, regulatory requirements for viral vector production, quality control, and compliance may also continue to evolve, requiring ongoing efforts to ensure compliance.

It's also worth noting that the feasibility and readiness of viral vector platforms may vary depending on the specific application, vector type, and production scale. Some viral vectors may require more complex and specialized production systems, while others may be more amenable to existing technologies. In either case, it is imperative to standardize your processes to ensure robustness, reproducibility, scalability and cost–effectiveness.

Continued research, development, and collaboration between academia, industry, and regulatory agencies will be key to advancing the field and ensuring that we are ready to build robust and scalable viral vector platforms.

DOI: 10.18609/cgti.2023.056

BIOGRAPHY

DR RAQUEL MARTÍN-IBÁÑEZ is a seasoned professional with over 10 years of experience in biotechnology and advanced therapy medicinal products (ATMPs). Her expertise lies in the development and manufacturing of cell and gene therapies, with a focus on translating these products into clinical application. Dr. Martín-Ibáñez has successfully led several translational projects, including the development of a dendritic cell vaccine for HIV and the production of genetically modified lentiviral particles for CAR19 therapy. She has also implemented a manufacturing process for the reprogramming of GMP-grade iPSC lines and the production of plasmids under GMP compliance. Dr Martín-Ibáñez holds a bachelor's and master's degrees in Pharmaceutical Sciences and a PhD in neuroscience. She started her career in the translational field in 2012 as a Qualified Person and Quality Assurance Manager at Creatio, the Production and Validation Center of Advanced Therapies at the University of Barcelona. In 2019, she joined Cedars-Sinai Medical Center to establish their new biomanufacturing facilities (CBC), where she played a key role in the successful launch of the center. She has recently taken on a new role at the Center for Cancer Cell Therapy at Stanford Medical School as Associate Director of Process Development and Manufacturing. Dr Martín-Ibáñez is committed to accelerating the clinical translation of gene and cell therapy products by applying process optimization principles from the earliest stages of development. With her drive and expertise, she aims to make a significant contribution to the field of gene and cell therapy products.

AFFILIATION

Raquel Martín-Ibáñez, PhD
Associate Director for Process Development and Manufacturing
Stanford Center for Cancer Cell Therapy, Stanford Cancer Institute, Stanford University, Stanford

REFERENCES

1. Bezeljak U. Cancer gene therapy goes viral: viral vector platforms come of age. *Radiol. Oncol.* 2022; 56(1), 1–13.
2. McCarron A, Donnelley M, McIntyre C, Parsons D. Challenges of up-scaling lentivirus production and processing. *J. Biotechnol.* 2016; 240, 23–30.
3. Minh AD, Kamen AA Critical Assessment of Purification and Analytical Technologies for Enveloped Viral Vector and Vaccine Processing and Their Current Limitations in Resolving Co-Expressed Extracellular Vesicles. *Vaccines (Basel)* 2021; 9(8), 823.
4. Perry C, Rayat Acme. Lentiviral Vector Bioprocessing. *Viruses* 2021; 13(2), 268.
5. Kasemiire A, Avohou HT, De Bleye C *et al.* Design of experiments and design space approaches in the pharmaceutical bioprocess optimization. *E. Eur. J. Pharm. Biopharm.* 2021; 166, 144–154.
6. Yu LX, Amidon G, Khan MA *et al.* Understanding pharmaceutical quality by design. *AAPS J.* 2014; 16(4), 771–783.
7. Ferreira MV, Cabral ET, Coroadinha AS. Progress and Perspectives in the Development of Lentiviral Vector Producer Cells. *Biotechnol. J.* 2021; 16(1), e2000017.
8. Lesch HP. Back to the future: where are we taking lentiviral vector manufacturing. *Cell Gene Ther. Insights* 2018; 4(11), 1137–1150.

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given her approval for this version to be published.

Acknowledgements: None.

Disclosures and potential conflicts of interest: The views and opinions expressed in this article are those of the Dr Martín-Ibáñez and do not necessarily reflect the views or positions of any entities she represents.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited.

Revised manuscript received: Apr 19 2023; **Publication date:** Apr 26 2023

DOI: 10.18609/cgti.2023.056

CELL & GENE THERAPY INSIGHTS

VECTOR BIOPROCESSING & RAW MATERIALS

SPOTLIGHT

COMMENTARY

Opportunities to implement continuous processing in production of recombinant adeno-associated viral vectors

Garima Thakur, Sheldon Mink, Hanne Bak & Andrew D Tustian

Shifting from batch to continuous manufacturing is a promising way of lowering manufacturing costs by 60–80%. This is because continuous processing allows upstream and downstream unit operations to run simultaneously with reduced downtime, higher productivity, and at several-fold smaller scale via a range of enabling technologies including perfusion bioreactors, single pass filtration modules, and multi-column chromatography systems. Most advancements in continuous processing have been made in the context of monoclonal antibody processes, but no end-to-end continuous process has yet been implemented at scale. This is in part due to a lack of business case supporting the creation of new continuous manufacturing facilities and processes versus utilization of existing legacy batch processing infrastructure with well-established norms. However, the business case for continuous processing is stronger for production of recombinant adeno-associated viral vectors (rAAV) due to high treatment costs and a paucity of existing manufacturing facilities. Gene therapy treatments based on rAAV currently cost up to USD 3 million per patient and have high cost-of-goods ranging up to USD 1 million per dose. Thus, continuous processing can play a critical role in making rAAV treatments more affordable and accessible. In this article, we explore recent developments in continuous processing for rAAV and provide an overview of some of the major opportunities for intensification of rAAV processes by applying continuous processing tools, many of which were originally developed for monoclonal antibodies but can be equally well or even better suited for rAAV production.

Cell & Gene Therapy Insights 2023 9(3), 563–579

DOI: 10.18609/cgti.2023.082

Recombinant adeno-associated viral vectors (rAAV) are currently the preferred mode of delivery for *in-vivo* gene therapy treatments [1]. Over 200 rAAV-based gene therapy clinical trials are currently ongoing, and five products have been approved and commercialized, including Luxturna (AAV2), Zolgensma (AAV9), Hemgenix (AAV5), Upstaza (AAV2), and Roctavian (AAV5) [2]. However, large-scale manufacturing of rAAV to support late-stage clinical trials and commercial production remains a significant challenge due to low production yields, limited scalability, and high facility and consumable costs [3]. The average cost-of-goods (COGs) for a gene therapy treatment is estimated to be in the range of US $ 0.5-1 million per patient, and the treatments themselves range from USD 0.8–3.0 million per patient [4]. Thus, it is evident that COGs are a significant contributor to the current price of rAAV treatments, that is likely to be out of reach for most patients that can benefit from treatment. This challenge must be addressed before rAAV products can move beyond ultra-rare indications and become accessible to larger patient populations, particularly in lesser developed economies.

Shifting from batch to continuous processing is a promising way to significantly lower manufacturing costs. Continuous processing has been extensively explored in the context of monoclonal antibody (mAb) manufacturing and has been estimated to result in 68% and 35% reduction of COGs per gram of mAb produced for clinical and commercial scales, respectively [5]. In a continuous process, there is a constant flow of material across all manufacturing unit operations running simultaneously [6]. This contrasts with batch-mode manufacturing processes, in which the material is first produced in a bioreactor and then processed sequentially through each batch unit operation, including harvest, clarification, tangential flow filtration, capture chromatography, viral inactivation, polishing chromatography, viral retentive filtration, and final formulation. Figure 1 and 2 illustrates a typical process for rAAV in terms of setup, instrumentation, and scheduling for batch and continuous modes of operation.

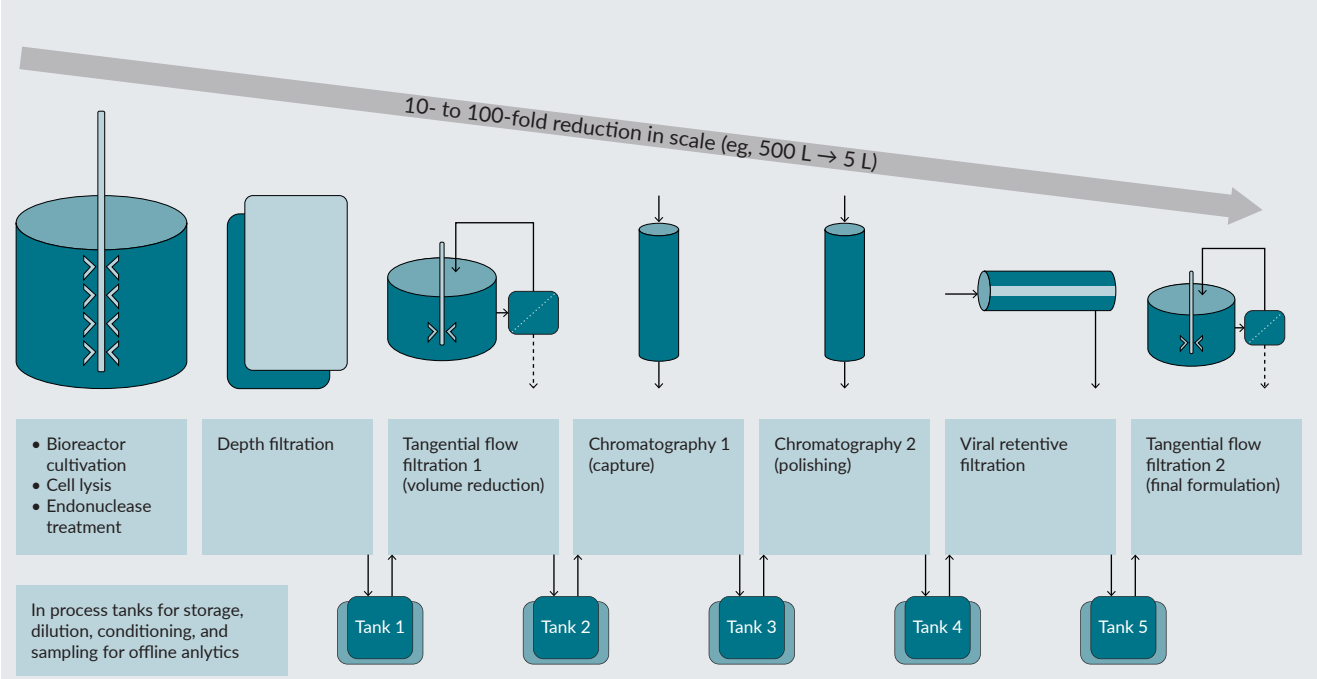
Both batch and continuous processing use the same sequence of unit operations, and the difference in COGs and productivity is driven by reduced downtime, smaller operating scales, higher equipment utilization rates, and higher volumetric productivity. Batch processing requires large-scale equipment sufficient to process an entire batch in one go, unlike continuous processing where smaller scales can be used as the material volume is spread out across all unit operations and across time. Furthermore, batch processing results in significant equipment down-time as all operations are idle during manufacturing except for the currently ongoing step, whereas all units operate non-stop in a continuous process. Options exist for intensification of batch operations such as via 'six-pack' manufacturing facilities in which six 12,000 L bioreactors are used to feed a single purification train [7]. In this case, equipment utilization rate is midway between the options shown in Figures 3A and 3B below. Batch mode operations can be converted to continuous mode by adapting the unit operations to continuously accept material inflow and outflow, and several technology enablers are available to achieve this, including perfusion or parallel batch bioreactor systems, single-pass filtration modules, multi-column chromatography systems, and twin-tank viral inactivation and formulation units [8].

Though this enabling technology has been developed in the context of mAb processes, it is are highly applicable to rAAV production, as the unit operations in both processes are based on the same principles of bioreactor production, packed bed chromatographic purification, depth filtration and membrane operations [9]. Moreover, there are several characteristics of rAAV processes, including low titers, short bioreactor production times, and multiple ultrafiltration steps, which are uniquely well-suited to continuous processing. Also, the economic driving force to reduce COGs and increase production volumes while avoiding scalability issues is much stronger in

DOI: 10.18609/cgti.2023.082

FIGURE 1

Setup and scheduling for batch processing of rAAV products.

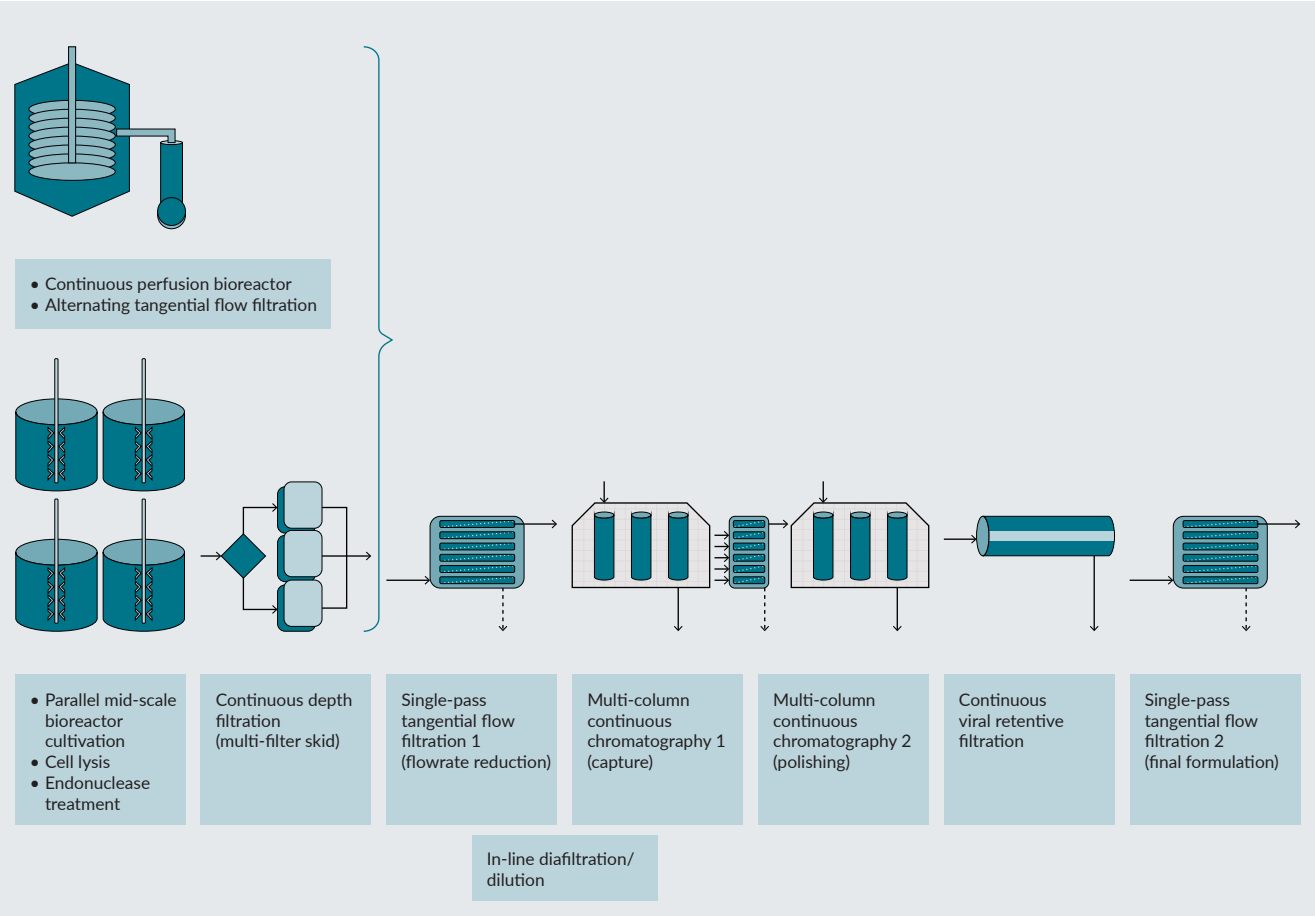


the case of rAAV compared to mAb processes due to the high costs of these therapies and the scale-up challenges which have not yet been resolved for many expression systems. Finally, as the rAAV production landscape is less established, the barrier to entry for new manufacturing approaches is lower than it is for mAbs. There are few existing commercial manufacturing facilities, particularly as it is challenging to convert existing mAb facilities to produce rAAV. This facility fit issue arises due to viral cross-contamination risks as well as the different scales involved, especially in the latter stages of operation where bioreactor production volumes are reduced 100- to 1000-fold for rAAVs compared to 10-fold for mAbs [10]. Thus, continuous processing can be realistically considered for future rAAV manufacturing facilities, unlike in the mAbs space where there is less of an economic driving force and more legacy infrastructure available, limiting the need to build new facilities or retrofit existing ones [11].

However, end-to-end continuous operation has not been implemented at scale for any biotherapeutics till date. Furthermore, only one product manufactured on a fully continuous platform has entered clinical trials, namely a biosimilar antibody produced by Australia-based company BiosanaPharma B. V. which reported successful Phase 1 results for the drug in March 2020 [12]. One reason for the lack of adoption of continuous processing till date is the need for high levels of automation, monitoring, and control across all unit operations simultaneously to compensate for any process deviations or drifts in input material to ensure that the critical quality attributes (CQA) of the final product are maintained within the established specifications. This requires specialized equipment, high levels of process digitalization, and rapid analytics [13]. However, the principles and key enablers of continuous processing, including perfusion cell culture, continuous multi-column chromatography, and single-pass tangential flow filtration, have been implemented by biopharmaceutical companies to intensify different sub-units of their manufacturing processes. In this article,

FIGURE 2

Setup and scheduling for continuous processing of rAAV products.



we explore recent developments in continuous processing for rAAV, as well as provide an overview of some of the major opportunities for intensification of rAAV processes by applying continuous processing tools, many of which were originally developed for mAbs but can be equally well or better suited to rAAV production.

CONTINUOUS UPSTREAM PROCESSING

Currently, the three most common rAAV manufacturing systems are transient transfection (TT) of HEK293 cells, baculovirus expression vector (BEV) systems used in Sf9 insect cells, and stable producer cell lines (PCL) [14]. TT systems are most common as they can be used to produce a wide range of rAAV constructs using different plasmids, and adherent TT (aTT) systems are commonly used in research laboratories due to their versatility and ease of use. However, aTT is challenging to scale up, driving up the popularity of suspension TT (sTT) systems that allow for volumetric scale-up [15]. BEV systems have been reported to be easier to scale-up than sTT systems, and their volumetric productivity in terms of viral particles produced can be greater than for mammalian cell-based systems [16]. However, due to inherent differences between mammalian and insect cells, there are structural differences in the product, including in the ratios

DOI: 10.18609/cgti.2023.082

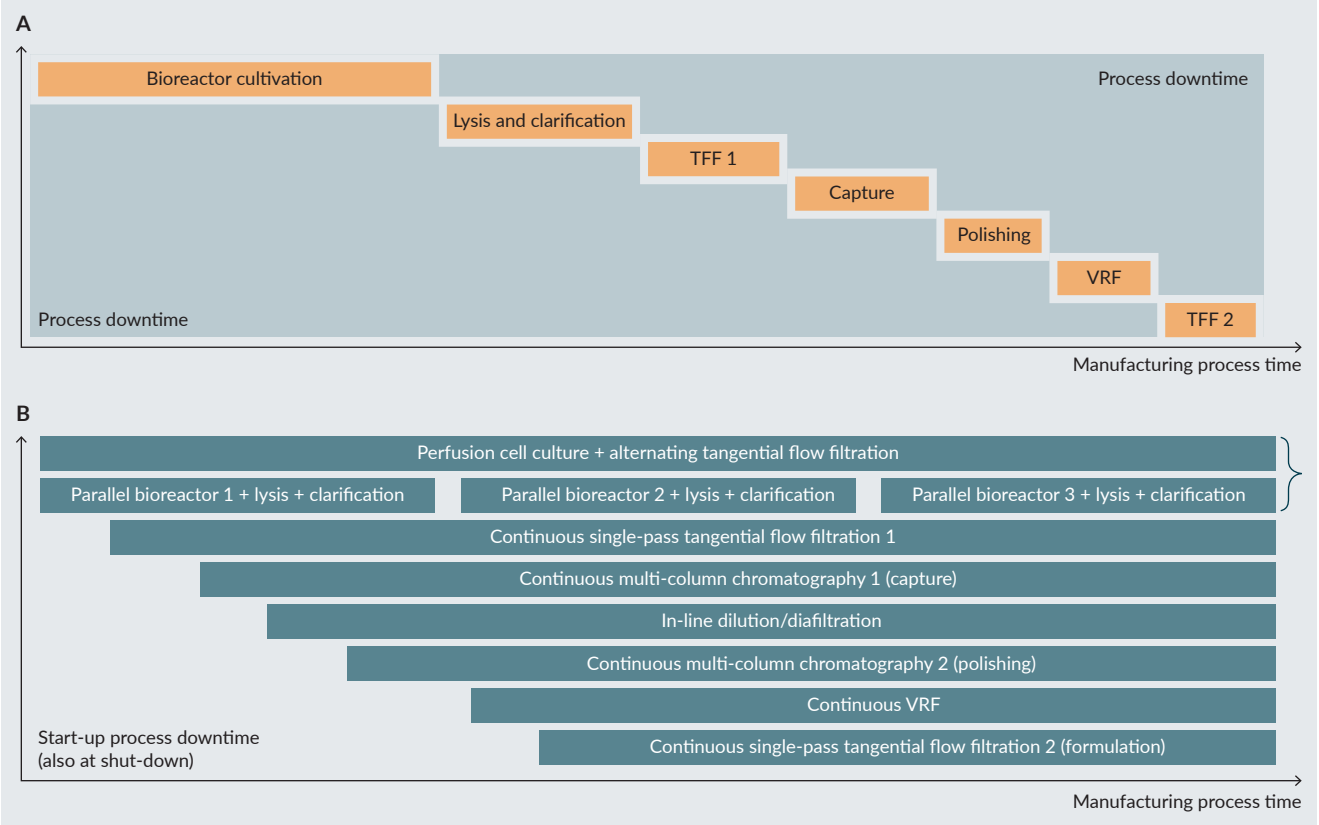
of the three viral proteins and post-translational modifications [17]. Finally, suspension PCL systems are ideal in their ease of scalability and batch-to-batch consistency, and 2,000 L batches have been recently demonstrated. However, it is a significant challenge to stably incorporate all the required genes in the correct configuration needed to produce viral proteins along with the required gene of interest while also ensuring correct assembly and packaging of the final viral particle, particularly as some of the required genes are cytotoxic, which limits the rapid development of these systems [18].

Scheduled multi-batch production integrated with continuous downstream operations

To integrate upstream operations into an end-to-end continuous process, there must either be continuous production of material, or multiple parallel batch bioreactors that are scheduled such that the remaining unit operations can be operated continuously with a constant inflow of material [19]. As seen in Figure 3A and 3B, the key requirement is an elimination of down-time between steps with continuous flow of upstream material through the downstream purification train. Complete continuity in upstream processes is not achievable, as cells by their nature require time to grow and expand before they can begin to produce the product. Moreover, unlike mAb or lentiviral production in which the product is secreted from the cells and can be removed from the bioreactor using a media perfusion system, rAAV is produced intracellularly except in limited cases, requiring cell lysis prior to further processing [20]. Another key challenge with rAAV bioreactors is that the cells only remain viable for 3–5 days

FIGURE 3

Setup and scheduling for batch (A) and continuous (B) processing of rAAV products



post-transfection, and thus cannot be kept alive for extended durations such as 30+ days as in the case of mAb therapeutics. Thus, a scheduled multi-batch approach is well-suited to continuous processing for rAAV, though advances have also been made towards fully continuous operations, as discussed in the next section.

With regards to scheduling, rAAV processes have an advantage over mAb processes as rAAV bioreactors are typically harvested 2–3 days post-transfection, unlike mAb bioreactors which are harvested on day 10–16 [21]. Thus, there is an opportunity to have faster cycling time with a lesser number of total mid-scale bioreactor systems, while still achieving constant supply of feed material to downstream. For example, mAb manufacturing facilities are able to eliminate the need for scale-up to 20,000 L for commercial manufacturing by having six parallel 2,000 L bioreactors operating with staggered harvest schedules. This can be considered continuous processing if the downstream train is receiving a continuous flow of material into continuous capture chromatography and subsequent purification steps, and these are able to operate without pause between bioreactor harvests [22]. Similar throughputs could likely be achieved for rAAV processes with only two to four parallel bioreactors due to the more frequent harvests.

Recent advancements in TT bioreactor technologies can help to overcome the challenges of large-scale operations including reductions in cell-specific productivities and difficulty in addition of time-sensitive transfection complexes. For aTT systems, a recent advancement is the development of the iCELLis fixed-bed bioreactor system which has been shown to lower plasmid DNA requirements by 20% and increase transfection efficiency by 20% compared to sTT systems at the 1,000 L scale [23]. Moreover, it is suggested to use more than one iCELLis system to increase throughput rather than scaling up beyond 1,000 L, which is ideal for continuous processing. There have also been several advances towards automated and integrated upstream production systems, such as the NevoLineTM system which integrates all upstream and midstream steps, including inoculation, upstream production, clarification, concentration and diafiltration to deliver a concentrated, clarified bulk ready for downstream processing [24]. The system is adaptable to aTT, sTT, and PCL systems and enables hands-off operation with integrated process steps in a single closed unit.

Overall, operating multiple automated bioreactor systems in parallel can be a key component of continuous rAAV manufacturing facilities by providing a steady stream of well-controlled material to the downstream train. Improvements in titer and productivity of rAAV bioreactors are also key enablers of continuous processing as they allow more material to be produced per unit time, allowing downstream operations to operate continuously without an upstream material bottleneck. Novel transfection reagents have been recently developed which have been shown to increase genomic and capsid titer 10- to 20-fold compared to the traditional polyethyleneimine (PEI) transfection regent [25]. Novel plasmids have also been engineered with optimized transfection ability in HEK293 cells, along with minimum generation of replication-competent rAAV [26]. Significant research is also ongoing on optimizing bioreactor parameters for higher titers and yield, including using different ratios of the transgene and packaging plasmids, and cultivating the bioreactor to higher cell densities [27].

Finally, it is important to note that PCL systems are particularly well-suited to parallel-batch continuous processing as they demonstrate the highest batch-to-batch consistency and do not require transfection. One such example is the a stable PCL system called ELEVECTA was recently developed in which a human amniocyte cell line (CAP® cells) are stably integrated with all components necessary for rAAV production, namely adenovirus helper functions E1A, E1B, E2A, E40RF6,

DOI: 10.18609/cgti.2023.082

VA RNA, as well as AAV replicase, rAAV8 capsid sequences and a gene of interest flanked by the AAV inverted terminal repeats [28]. As some of the integrated components are cytotoxic, expression is regulated by a doxycycline inducible promoter. The ELEVECTA system is well suited for continuous facilities as it does not rely on continuous availability of cGMP plasmids, a potential supply bottleneck which can put continuous operations at risk. The system is also scalable up to 2,000 L scale and has high yield in the order of 5e13 viral genomes/L. However, due to the scientific and technical challenges of developing PCL systems, it remains to be seen whether such approaches will become the norm for rAAV manufacturing as they are for mAbs.

Fully continuous upstream systems

Fully continuous upstream production systems require constant outflow of material from the production bioreactor prior to lysis, in contrast to the former approach in which staggered scheduled batch or fed-batch harvests are used to feed a continuous downstream purification train. This has been achieved for mAbs in the form of perfusion cell culture systems in which cells are grown to high densities and supernatant containing the mAb product as well as spent media is continuously removed from the bioreactor while cells are retained [29]. This is accomplished using alternating tangential-flow filtration (ATF) combined with continuous feed of fresh media in a 'feed and bleed' approach [30]. Such systems are only applicable to rAAV processes in which the viral particles are predominantly or exclusively secreted extracellularly, which are relatively rare. Furthermore, as mentioned previously, the key challenge with rAAV bioreactors is that the cells only remain viable for 3–5 days post-transfection, and thus cannot be kept alive for extended durations such as 30+ days as in the case of mAb therapeutics.

A recent example of perfusion in rAAV production is the ELEVECTA system discussed in the preceding section, in which quantification of viral genome concentration from 2–6 days post-induction showed that over 90% of the total viral particles were in the supernatant from day four onwards [28]. The product concentration was in the order of 5e13 viral genomes/L and was consistent across scales from 10 L to 200 L. This provides an opportunity for a 3-day window of continuous harvest via a perfusion ATF system, like the 10-day window in mAb processing, with material flowing continuously out of the bioreactor and into downstream processing train. The ELEVECTA system was also demonstrated with N-1 perfusion, which is an approach utilizing continuous expansion of cell lines by attaching a cell retention device to the N-1 bioreactor to attain high cell density and viability, allowing the N bioreactor to be seeded at a higher starting cell density and shortening the production time [31]. The ELEVECTA cells were initially grown in batch mode, after which perfusion was initiated to enable growth to high cell density, which was 5-fold higher at the time of induction than in the batch-mode process. Furthermore, overall volumetric production for rAAV was 40-fold higher in perfusion yield, leading to 8-fold higher cell-specific yield with 30–40% full particles [28]. Another benefit of N-1 perfusion is that a single bioreactor could provide a continuous supply of seed to multiple N bioreactors, facilitating robust parallel-batch continuous processing.

Another example of perfusion was demonstrated for rAAV8 and rAAV9 production in a 2 L WAVE bioreactor, in which a 1 L cell culture was transfected and 80% of the media was harvested through a perfusion filter and replaced with fresh media every 24 h from 48- to 144-h post-transfection [21]. The viral genome yield recovered from the harvested media across all time-points was 92% of the total process yield, with the yield from the lysed pellet at 144 h comprising only 8%. Overall, the approach resulted in a 6.5-fold and 4.8-fold increase in the yield of rAAV8 and rAAV9, respectively. Though this has only been demonstrated at

lab-scale till date, the approach is promising for continuous processing. Such perfusion production systems also have other advantages as there is no need for cell lysis which introduces additional host cell proteins and host cell DNA into the feed stream, burdening downstream processing and requiring additional processing steps including endonuclease treatment and depth filtration, all of which increase COGs. Overall, this approach represents the ideal situation for continuous processing, as the perfusate can be fed directly into downstream unit operations with overall enhanced process yields even without lysis, though it is not yet realistic to expect such high yields of fully packaged rAAV directly in the bioreactor supernatant for all serotypes or even for rAAV8 and rAAV9 processes.

Finally, a recent proof-of-concept explored the potential for using continuous processing principles in the bioreactor transfection step to overcome the variability seen with manual transfections at scale [32]. It was demonstrated that a 2,000 L suspension bioreactor would require 500 L of PEI:DNA complex, and that there were uneven mixing effects in the transfection tank prior to addition, which led to a wide distribution in the size of transfection complexes and adversely affected transfection efficiency. An automated continuous transfection system was developed in which cells were grown in a stirred-tank suspension bioreactor, after which they were anchored on microcarriers prior to automated addition of transfection complexes. The continuous system used automated mixing of microcarriers with HEK293 cells as well as parallel mixing of transfection reagent and plasmid DNA, prior to automated mixing of all four components after fixed incubation time to control the growth of the transfection complex. This operation was repeated seven times over 20 days, and the continuous transfection approach was shown to effectively achieve a narrow size distribution of transfection complex across the upstream process.

CONTINUOUS DOWNSTREAM PROCESSING

The downstream continuous processing landscape is rich in tools that have been developed for mAbs, enzymes, and microbial biotherapeutics. For continuous clarification, options used include ATF, continuous depth filtration using multi-filter skids, and continuous centrifugation [33]. For continuous chromatography, versatile equipment setups have been commercialized with in-built pumps, valves, and sensors enabling integrated multi-column continuous operation, including the BioSMB (Sartorius), Akta PCC (Cytiva), Octave SMB (Tarpon), Contichrom CUBE (Chromacon), and BioSC (Novasep) systems [34]. Single-use, column free systems have also been proposed, such as Continuous Counter-Current Tangential Chromatography (CCTC) [35]. Continuous viral clearance has been shown via continuous viral retentive filtration as well as via continuous pH or detergent-based viral inactivation using twin-tank systems or coiled flow reactors [36]. Finally, continuous formulation has been achieved using single-pass tangential flow filtration modules for in-line concentration and in-line diafiltration of the process material [37].

Converting these unit operations from batch to continuous mode for mAbs has been shown to result in smaller operating scales, improved utilization of consumables including resins and membranes, savings in buffer volumes, lower manpower requirements, lower residence time of the biotherapeutic in-process, and the ability to achieve steady-state operation across long continuous campaigns without batch-to-batch variability in critical process parameters (CPP) and critical quality attributes (CQA) [13]. These advantages can also apply to rAAV production, as the rAAV process comprises similar unit operations including clarification, initial concentration, affinity chromatography, polishing chromatography, viral retentive filtration, and formulation. Different enabling technology is used at each step in the process

DOI: 10.18609/cgti.2023.082

to convert the operation from batch to continuous mode, as illustrated in Figure 1 and 2. Of these, continuous ultrafiltration and continuous chromatography are the most critical and are discussed further below.

Continuous single-pass tangential flow ultrafiltration

Single-pass tangential flow filtration (SPTFF) is a key approach used for converting ultrafiltration and diafiltration (UF-DF) steps from batch to continuous mode [37]. In batch mode, UF-DF is carried out by storing the process material in a large tank and recirculating it across an ultrafiltration membrane such that the biotherapeutic molecule is retained on the retentate side and the buffer passes into the permeate stream, facilitating concentration and/or buffer exchange. This operation is difficult to integrate into a continuous setup due to long processing times and the need to concentrate inside a retentate vessel, which does not allow continuous inflow or outflow of material from the unit operation. SPTFF overcomes this problem by essentially replacing the single membrane module with an internally staged series of membranes with overall several-fold higher membrane area, enabling high volumetric concentration factors in a single pass of the process material across the SPTFF module without the need for recirculation [38]. Diafiltration using SPTFF is also possible with multi-inlet SPTFF modules available in which process material and diafiltration buffer can be fed simultaneously, achieving >99% buffer exchange in a single pass [39].

Downstream rAAV processes stand to benefit significantly from SPTFF technology. Firstly, low titers from upstream typically necessitate 10-fold concentration prior to affinity chromatography to avoid long loading times. Additionally, diafiltration of the harvest material prior to capture has been shown to improve removal of host cell proteins and potentially oncogenic host cell DNA fragments which would otherwise be loaded onto the affinity column along with the target rAAV. Converting this step from batch to continuous mode would allow clarified material to be loaded directly onto an affinity chromatography column after a single pass across an SPTFF module, effectively integrating these two unit operations into a single step and reducing processing time, complexity, and footprint. Similar pre-affinity concentration steps have been demonstrated for mAb processes and have been shown to result in significant productivity enhancements for the affinity capture step due to the more concentrated load [40]. This step is non-essential in the case of mAb processing due to high bioreactor titers with no need for further concentration prior to capture. Thus, adding SPTFF at this point in the process is an optional extra enhancement for mAb process, but more significant for rAAV where the operation is carried out even in the base process.

Another advantage of using SPTFF for concentration of low-titer rAAV process streams is that high volumetric concentration factors of 10–50× can be readily achieved, as showcased in a recent case study for 50× concentration of rAAV9 using SPTFF post-harvest [41]. For example, an rAAV titer of 1×10^{15} capsids/L is equivalent to only 0.065 g/L of protein, in stark contrast to mAb processes where titers range from 1–10 g/L and final doses can exceed 200 g/L. Operating at low concentrations leads to exceptionally high fluxes in SPTFF processes as there is low viscosity and little to no concentration polarization effects reducing the permeate flux [42]. Thus, SPTFF can be readily deployed for both in-process UF-DF as well as in the final formulation step without the need for complex flux control systems with permeate and retentate pumps and/or valves, which are typically required in mAb processing to achieve high concentrations. Furthermore, modular SPTFF kits are available in which the internally staged configuration can be adjusted as needed, with increasing serial stages used for achieving higher concentration

factors, while increasing parallel stages facilitate higher volumetric throughput. Thus, customized assemblies can be configured to suit both the initial large-scale volume reduction in rAAV processes post-harvest and the low-volume final formulation step.

Continuous chromatography

Chromatography steps can be considered continuous if there is either continuous loading of input material onto the columns, continuous outflow of eluted material to the next unit operation, or both [43]. As conventional chromatography is by nature a periodic unit operation, with a single column cycling through equilibration, loading, wash, elution, and cleaning phases, the most common way to achieve continuity is by increasing the number of columns operating in parallel, with one or more columns in the 'loading' phase while the others cycle through 'non-loading' steps of the same total duration [44]. Alternative approaches to continuous chromatography include re-engineering the chromatography setup such as by continuously recirculating resin beads across a flow path in continuous counter-current tangential chromatography, or using continuous annular chromatography in which mobile and stationary phases move across each other concurrently by spinning [35]. However, despite advantages in productivity and the true continuous nature of these systems, these are not widely accepted in the industry, unlike multi-column chromatography which has already been implemented at manufacturing scale by several large biopharmaceutical manufacturers for the affinity chromatography step in mAb processing.

Continuous affinity capture chromatography

The main benefit of continuous multi-column affinity chromatography is reduced column sizes and lower resin requirements, along with increased productivity in terms of both g protein/day as well as g protein/mL resin [5]. The driving factors behind these improvements are twofold. Firstly, there is no need to process the entire batch in one or two chromatography cycles, as the material inflow is spread out continuously across time and across all other unit operations. Thus, columns can be much smaller and are sized according to the overall continuous process flow rate. Secondly, in batch mode, columns are loaded until 2–5% breakthrough to prevent loss of material. However, a well-established technique in continuous processing is to connect two columns in series during the loading step, thus enabling the first column to be loaded up to 70–80% breakthrough as the second column is in place to capture any breakthrough material. Using this technique, binding capacities of mAb affinity capture resins have been shown to increase significantly, for example from 35 g/L at 1% breakthrough to 50–60 g/L at 70% breakthrough for mAbSelect SuRe resin in recent case studies [45,46].

Similar or better COGs reduction can be expected for continuous capture in rAAV processes, as affinity resins including Capto AVB and Poros CaptureSelect AAV resins are 30-200% more expensive than mAb affinity ligands. Furthermore, in rAAV processes, column volumes are driven down by the concentration step prior to capture, lowering the process flow rate and requiring less volume to achieve the desired residence time for loading. For example, a 200 L upstream bioreactor with titer in the order of 1×10^{15} capsids/L harvested daily would result in a flow rate of only 14 mL/min into capture chromatography post-10× concentration, requiring a column volume of 35 mL to achieve the required 2.5-minute loading residence time. The total loading time would be 25 minutes given a resin binding capacity of 1×10^{17} capsids/L resin, and a total of two to three columns in parallel would result in a total resin requirement of 70–105 mL and would be sufficient to process the entire 200 L of material over the course of 24 h of continuous operation. In contrast, if this

DOI: 10.18609/cgti.2023.082

material was to be processed in batch mode in a single cycle, the required resin volume considering the same binding capacity of 1×10^{17} capsids/L resin would be 2 L. This is a >90% reduction in resin requirements, even if the smaller columns are periodically replaced to limit the total number of cycles per column. Further COGs reduction can be driven by using three rather than two affinity columns to enable higher binding capacities to be achieved by using two columns in series during loading.

Continuous polishing chromatography

Though continuous chromatography has been mainly implemented for affinity capture, several groups have demonstrated continuous polishing chromatography for mAb processes including anion exchange, cation exchange, mixed-mode, and hydrophobic interaction chromatography steps. Two key approaches are parallel multi-column operation on systems such as the Akta PCC or BioSMB, and multi-column counter-current solvent gradient purification (MCSGP) systems. In the former approach, two or more columns are operated in parallel as discussed in the preceding section, with a single elution step and/or gradient running on the system at any given time with volume-, conductivity-, or UV-based fractionation [34,47]. In the latter approach, elution is integrated with automated side-cut recycling and in-line dilution between two or more identical columns to enrich for the target species and enable removal of the unwanted variants across multiple columns with overall higher yield and purity than possible in a single-column process [48]. In the context of rAAV purification, both approaches are promising for separations of full and empty capsids.

The parallel multi-column approach is operationally simpler and enables batch operations to be directly converted to continuous mode without any adjustments required in the process method, simply by increasing the number of columns to two or more such that loading of the incoming material from the previous unit operation can be handled continuously. Thus, existing full/empty separation methods using anion exchange chromatography on resins such as Capto Q and POROS HQ, as well as on monoliths such as CIM QA, can be directly transferred to the continuous multi-column system [49]. BioSMB systems are particularly advantageous as they have a unique manifold configuration with 240 valves, seven pumps, and 16 column positions, and can carry out both capture and polishing chromatography steps on the same system as has been demonstrated in several case studies for mAbs [50]. A typical rAAV chromatography process consisting of affinity capture followed by full/empty separation in step or linear gradient can thus be executed on a single BioSMB system in continuous mode. Alternatively, MCSGP-based polishing strategies can also be explored to potentially achieve higher enrichment of full capsids, though independent chromatography systems would be required for continuous capture and polishing in this case.

NEED FOR IMPROVED PROCESS ANALYTICAL TECHNOLOGY (PAT) TOOLS

One of the challenges in continuous operation which need to be addressed in the context of rAAV production is the lack of rapid and reliable analytical tools that can be deployed automatically over long continuous campaigns. As in the case of mAb processes, there is a need for rapid analytics executed in real time or near-real time that can track the critical process parameters (CPP) and critical quality attributes (CQA) across all continuous processing steps to monitor for deviations and respond by triggering operator alarms or executing predetermined control strategies [13]. Process analytical technology (PAT) monitoring and tools have been developed for mAb processes, including spectroscopic sensors based on Raman/IR spectroscopy [51], rapid at-line HPLC methods [52], and

statistical process controls [53] for tracking CPP and ensuring that they remain within the design space established for steady-state continuous operation in line with the principles of Quality by Design [54]. Additionally, work has been done on end-to-end process integration using digital control systems combined with level-controlled surge tanks that act as potential breakpoints between unit operation for additional process robustness [50,55,56]. However, implementation of these techniques is limited to lab or pilot scale in the mAbs space, and such development is largely absent for rAAV processes [57].

It is important to note not all methods used for final product release are required for in-process control during manufacturing, and thus limited measurements of key CQA including capsid titer, empty/full capsid ratio, genomic titer, and % of aggregates and fragments are the primary CQA measurements likely required in continuous operation, with other CQA including infectivity, replication competency, and residuals including HCP, HCDNA, plasmid DNA, endonuclease, transfection reagent, and/or affinity ligand required only at the final DS stage [58]. Automated approaches for sampling, measurement and data analysis of ddPCR [59] and ELISA [60] assays for quantification of genomic and capsid titer are a critical first step. Additionally, assays for quantification of genomic and capsid titer are a critical first step. Both are a current focus in the industry. Furthermore, mass photometry has emerged as a rapid tool for quantification of full/empty ratio with measurement times in the range of 2–5 minutes, and this has high potential to be deployed in continuous processing if the manual sample prep step can be automated [61]. Finally, in-line dynamic light scattering, multi-angle light scattering, and fluorescence measurements have been shown to be effective for monitoring rAAV CQA and can be deployed for real-time analytics in a continuous process, particularly when combined with at-line HPLC methods [62,63].

Lastly, a key requirement of continuous processing is the need for concentration sensors to monitor the rAAV in the input and output process streams from each unit operation in near-real time [53]. These concentration measurements are critical for many downstream unit operations as the loading onto columns, membranes and filters is a CPP affecting performance, resolution, and/or yield. The need for this measurement is exacerbated in case of titer variability in the upstream process, or variability in the in-process streams due to deviations or fluctuations in any of the preceding unit operations [64]. Additionally, concentration-based feedback control is critical in SPTFF processes, particularly if SPTFF is used for the final formulation step, as it is critical to track and control the concentration of rAAV in the final lot. Several tools have been developed for in-line concentration measurements in mAb processes and have been integrated into feedback control loops for chromatography and SPTFF, including in-line single- and multi-wavelength UV [65], and infrared spectroscopy [66]. Similar sensors and tools need to be developed for rAAV processes to facilitate monitoring and control of continuous processes. Overall, though there have been promising advances in rAAV analytics that have the potential to be converted into PAT tools, more work is required to establish these technologies in the context of continuous processing [18,67].

CONCLUSION

In conclusion, continuous processing offers significant opportunities for process improvement and intensification in rAAV manufacturing. Though most continuous processing developments are in the mAb space, essential process similarities between mAb and rAAV processes enable direct transfer of many of these tools and techniques including parallel-batch bioreactors or perfusion systems, single-pass tangential flow ultrafiltration for in-line concentration,

DOI: 10.18609/cgti.2023.082

diafiltration, and final formulation, and multi-column chromatography for affinity capture and full/empty separations. Moreover, many of the unique challenges of rAAV processes, including shorter bioreactor production times, low titers, and the need for multiple concentration steps, can be particularly well-handled by continuous processing. Furthermore, continuous processing can reduce the scale-up challenges in upstream bioreactors by enabling processes to be scaled-out across multiple bioreactors at 500–2,000 L scale, instead of scaled-up to 20,000 L where cell growth and transfection kinetics are difficult to control. Continuous processing also reduces the scale imbalance between the initial and final unit operations when the material is concentrated 100- or 1000-fold to reach the target concentration required for reasonable dosage volumes.

The business case for continuous processing is perhaps more compelling for rAAV than for mAb processes for two key reasons: The first is the cost of rAAV production, translating into list prices of millions of dollars for the patient. This not only limits the availability and affordability of rAAV products, but also attracts censure especially in the case of life-saving therapies. Thus, the reduction in COGs afforded by continuous processing is a major advantage. The second reason is the paucity of existing rAAV manufacturing plants and the difficulty of converting mAb facilities to rAAV due to the differences in scale and the risks of viral cross-contamination. Thus, there is a need to implement modular facilities adaptable to both clinical and commercial production of rAAV. This allows biopharmaceutical companies to genuinely consider the pros and cons of integrated continuous operation, without being held back by the need to utilize existing legacy infrastructure or the challenges of redefining well-established batch processing norms, as is common in the mAbs space.

AFFILIATION

Garima Thakur
Preclinical Manufacturing and Process Development,
Regeneron Pharmaceuticals Inc.

Sheldon Mink
Preclinical Manufacturing and Process Development,
Regeneron Pharmaceuticals Inc.

Hanne Bak
Preclinical Manufacturing and Process Development,
Regeneron Pharmaceuticals Inc.

Andrew D Tustian
Preclinical Manufacturing and Process Development,
Regeneron Pharmaceuticals Inc.

REFERENCES

1. Alliance for Regenerative Medicine. Project A-Gene A case study-based approach to integrating QbD principles in Gene Therapy CMC programs. 2021.
2. Shen W , Liu S, Ou L. rAAV immunogenicity, toxicity, and durability in 255 clinical trials: A meta-analysis. *Front. Immunol.* 2022; 13, 1001263.
3. Capra E, Gennari A, Loche A, Temps C. Viral-vector therapies at scale: Today's challenges and future opportunities. Mar 29 2022.
4. Macdonald GJ. Vector Production Processes Play Key Role in High Gene Therapy Prices. Genet. Eng. Biotechnol. News. Nov 22 2022.
5. Gupta P, Kateja N, Mishra S, Kaur H, Rathore AS. Economic assessment of continuous processing for manufacturing of biotherapeutics. *Biotechnol. Prog.*, 2021; 37(2), e3108.
6. Konstantinov KB, Cooney CL. White paper on continuous bioprocessing May 20–21 2014 continuous manufacturing symposium. *J. Pharm. Sci.* 2015; 104(3), 813–820.

7. Sinclair A. Disposable Bioreactors: The Next Generation. BioPharm Int. 2008; 21(4). (Accessed Apr 13 2023).

8. Arnold L, Lee K, Rucker-Pezzini J, Lee JH. Implementation of fully integrated continuous antibody processing: effects on productivity and COGm. *Biotechnol. J.* 2019; 14(2), 1800061.

9. Adams B, Bak H, Tustian AD. Moving from the bench towards a large scale, industrial platform process for adeno-associated viral vector purification. *Biotechnol. Bioeng.* 2020; 117(10), 3199–3211.

10. Hebben M. Downstream bioprocessing of AAV vectors: Industrial challenges \& regulatory requirements. *Cell Gene Ther. Insights* 2018; 4(2), 131–146.

11. Rathore AS, Thakur G, Kateja N. Continuous integrated manufacturing for biopharmaceuticals: A new paradigm or an empty promise? *Biotechnol. Bioeng.* 2023; 120(2), 333–351.

12. Vuksanaj K. First mAb Produced via Fully Continuous Biomanufacturing. Genet. Eng. Biotechnol. News (Sep 24, 2019). (Accessed Apr 14 2023).

13. The National Institute for Innovation in Manufacturing Biopharmaceuticals. N-mAb: A case study to support development and adoption of integrated continuous bioprocesses for monoclonal antibodies. 2022, 1–256.

14. Shupe J, Zhang A, Odenwelder DC, Dobrowsky T. Gene therapy: challenges in cell culture scale-up. *Curr. Opin. Biotechnol.* 2022; 75, 102721.

15. Dobrowsky T, Gianni D, Pieracci J, Suh J. AAV manufacturing for clinical use: insights on current challenges from the upstream process perspective. *Curr. Opin. Biomed. Eng.* 2021; 20, 100353.

16. Wu Y, Mei T, Jiang L, Han Z, Dong R, Yang T, Xu F. Development of Versatile and Flexible Sf9 Packaging Cell Line-Dependent OneBac System for Large-Scale Recombinant Adeno-Associated Virus Production. *Hum. Gene Ther. Methods* 2019; 30(5), 172–183.

17. Kondratov O, Marsic D, Crosson SM *et al.* Direct Head-to-Head Evaluation of Recombinant Adeno-associated Viral Vectors Manufactured in Human versus Insect Cells. *Mol Ther.* 2017; 25(12), 2661–2675.

18. Escandell JM, Pais DA, Carvalho SB, Vincent K, Gomes-Alves P, Alves PM. Leveraging rAAV bioprocess understanding and next generation bioanalytics development. *Curr. Opin. Biotechnol.* 2022; 74, 271–277.

19. Subramanian G, Bisschops M, Schofield M, Grace J. (2017). Two Mutually Enabling Trends: Continuous Bioprocessing and Single-Use Technologies. In Continuous Biomanufacturing - Innovative Technologies and Methods. (Editor: Subramanian G).

20. Vandenberghe LH, Xiao R, Lock M, Lin J, Korn M, Wilson JM. Efficient serotype-dependent release of functional vector into the culture medium during adeno-associated virus manufacturing. *Hum. Gene Ther.* 2010; 21(10), 1251–1257

21. Grieger JC, Soltys SM, Samulski RJ. Production of Recombinant Adeno-associated Virus Vectors Using Suspension HEK293 Cells and Continuous Harvest of Vector From the Culture Media for GMP FIX and FLT1 Clinical Vector. *Mol. Ther.* 2016; 24(2), 287–297.

22. Enright M. Amgen's new $200M next-gen biologic plant a first in U.S. Providence Business News. 2020.

23. Pall Corporation. Reducing Cost of Gene Therapy Commercialization. Pall Corporation. (Accessed Apr 03 2023).

24. Univercells Technologies. NevoLine™ platform. (Accessed Apr 03 2023).

25. Hebben M, Nyamay'antu A. Fecto-VIR-AAV Transfection Reagent. BioProcess Int. (Oct 29 2021). (Accessed Apr 06 2023).

26. Yin J. Achieving rAAV Bioreactor Titer Of >1E15 vg/L With Novel Plasmid Transient Transfection Platform. Bioprocess online. (Accessed Apr 06 2023).

27. Zhao H, Lee KJ, Daris M *et al.* Creation of a High-Yield AAV Vector Production Platform in Suspension Cells Using a Design-of-Experiment Approach. *Mol. Ther. Methods Clin. Dev.* 2020; 18, 312–320.

28. Coronel J, Patil A, Al-Dali A, Braβ T, Faust N, Wissing S. Efficient Production of rAAV in a Perfusion Bioreactor Using an ELEVECTA® Stable Producer Cell Line. Genet. Eng. Biotechnol. News 2021.

29. Bielser JM, Wolf M, Souquet J, Broly H, Morbidelli M. Perfusion mammalian cell culture for recombinant protein manufacturing-A critical review. *Biotechnol. Adv.* 2018; 36(4), 1328–1340.

30. Clincke MF, Mölleryd C, Samani PK, Lindskog E, Fäldt E, Walsh K, Chotteau V. Very high density of Chinese hamster ovary cells in perfusion by alternating tangential flow or tangential flow filtration in WAVE Bioreactor™-part II: Applications for antibody production

DOI: 10.18609/cgti.2023.082

and cryopreservation. *Biotechnol. Prog.* 2013; 29(3), 768–777.

31. Dobrowsky T, Shupe JW. Seed culture process for aav production WO2020154607A1. (Jul 30 2020). (Accessed: Apr 06 2023).

32. Ladd B, Bowes K, Lundgren M, Gräslund T, Chotteau V. Proof-of-concept of continuous transfection for adeno-associated virus production in microcarrier-based culture. *Processes*, 2022; 10(3), 515.

33. Gupta A, Amara JP, Gousseinov E, Cacace B. Recent advances in harvest clarification for antibodies and related products. *Approaches Purif. Anal. Charact. Antib.-Based Ther.* 2020,117–136.

34. Kateja N, Tiwari A, Thakur G, Rathore AS. Complete or periodic continuity in continuous manufacturing platforms for production of monoclonal antibodies? *Biotechnol. J.* 2021; 16(7), e2000524.

35. Dutta AK, Fedorenko D, Tan J, Costanzo JA, Kahn DS, Zydney AL, Shinkazh O. Continuous countercurrent tangential chromatography for mixed mode post-capture operations in monoclonal antibody purification. *J. Chromatogr A.* 2017; 1511, 37–44.

36. Thakur G, Ghumade P, Rathore AS. Process analytical technology in continuous processing: Model-based real time control of pH between capture chromatography and viral inactivation for monoclonal antibody production. *J. Chromatogr. A*, 2021; 1658, 462614.

37. Casey C, Gallos T, Alekseev Y, Ayturk E, Pearl S. Protein concentration with single-pass tangential flow filtration (SPTFF). *J. Membr. Sci.* 2011; 384(1–2), 82–88.

38. Arunkumar A, Singh N, Peck M, Borys MC, Li ZJ. Investigation of single-pass tangential flow filtration (SPTFF) as an inline concentration step for cell culture harvest. *J. Membr. Sci.* 2017; 524, 20–32.

39. Pall-Biotech. Performance of the Cadence™ Inline Concentrator Modules for the Concentration of Human IgG. *Appl. NoteUSD 3309*, 2015.

40. Casey C, Rogler K, Gjoka X, Gantier R, Ayturk E. Cadence™ Single-pass TFF Coupled with Chromatography Steps Enables Continuous Bioprocessing while Reducing Processing Times and Volumes. *Appl. Note*, 1–7, 2015.

41. Purification of adeno-associated virus by single-pass tangential flow filtration (SPTFF) (Aug 22 2021). (Accessed Apr 03 2023).

42. Thakur G, Thori S, Rathore AS. Implementing PAT for single-pass tangential flow ultrafiltration for continuous manufacturing of monoclonal antibodies, *J. Membr. Sci.* 2020; 613, 118492.

43. Coffman J, Lin H, Wang SB *et al.* Balancing continuous, integrated, and batch processing. 2017.

44. Holzer M, Osuna-Sanchez H, David L. Multicolumn chromatography. *BioProcess Int.* 2008; 6(8), 74–84.

45. Warikoo V, Godawat R, Brower K *et al.* Integrated continuous production of recombinant therapeutic proteins. *Biotechnol. Bioeng.* 2012; 109(12), 3018–3029.

46. Thakur G, Bansode V, Rathore AS. Continuous manufacturing of monoclonal antibodies: Automated downstream control strategy for dynamic handling of titer variations. *J. Chromatogr. A.* 2022; 1682, 463496.

47. Feidl F, Vogg S, Wolf M *et al.* Process-wide control and automation of an integrated continuous manufacturing platform for antibodies. *Biotechnol. Bioeng.* 2020; 117(5), 1367–1380.

48. Krättli M, Müller-Späth T, Morbidelli M. Multifraction separation in countercurrent chromatography (MCSGP). *Biotechnol. Bioeng.* 2013; 110(9), 2436–2444.

49. Joshi PRH, Bernier A, Moço PD, Schrag J, Chahal PS, Kamen A. Development of a scalable and robust AEX method for enriched rAAV preparations in genome-containing VCs of serotypes 5, 6, 8, and 9. *Mol. Ther. Methods Clin. Dev.* 2021; 21, 341–356.

50. Thakur G, Nikita S, Tiwari A, Rathore AS. Control of surge tanks for continuous manufacturing of monoclonal antibodies. *Biotechnol. Bioeng.* 2021; 118(5), 1913–1931.

51. Bakeev KA. Process analytical technology: spectroscopic tools and implementation strategies for the chemical and pharmaceutical industries. *John Wiley & Sons* 2010.

52. Tiwari A, Kateja N, Chanana S, Rathore AS. Use of HPLC as an Enabler of Process Analytical Technology in Process Chromatography. *Anal. Chem.* 2018; 90(13), 7824–7829.

53. Manser B, Bisschops M. Technical Regulatory Topic-Automation and Control in Continuous Bioprocesses. 2021.

54. Rathore AS. Roadmap for implementation of quality by design (QbD) for

biotechnology products. *Trends Biotechnol.* 2009; 27(9), 546–553.

55. Chen Y, Yang O, Sampat C, Bhalode P, Ramachandran R, Ierapetritou M. Digital twins in pharmaceutical and biopharmaceutical manufacturing: a literature review. *Processes* 2020; 8(9), 1088.

56. Johnson MD, May SA, Mc Clary Groh *et al.* Understanding residence time, residence time distribution, and impact of surge vessels in *Continuous pharmaceutical processing*, Springer, 2020, 51–85.

57. Cashen P, Manser B. Quality by Design (QbD) for Adeno-Associated Virus (AAV) A Framework for a QbD Assessment for AAV Products Within the Chemistry Manufacturing and Controls (CMC) Documentation. *Pall Biotech White Pap.* 2021, 1–33.

58. Tustian AD, Bak, H. Assessment of quality attributes for adeno-associated viral vectors. *Biotechnol. Bioeng.* 2021; 118, 4186–4203.

59. Brink BG, Meskas J, Brinkman RR. ddPCRclust: an R package and Shiny app for automated analysis of multiplexed ddPCR data. *Bioinformatics* 2018; 34(15), 2687–2689.

60. BPI contributer/ Automated AAV8 Capsid Titer Assay Development - BioProcess InternationalBioProcess International. (Dec 10 2021). (Accessed Apr 03 2023).

61. Wu D, Hwang P, Li T, Piszczek G. Rapid characterization of adeno-associated virus (AAV) gene therapy vectors by mass photometry. *Gene Ther.* 2022; 29(12), 691–697.

62. Gagnon P, Goricar B, Mencin N, Zvanut T, Peljhan S, Leskovec M, Strancar A. Multiple-Monitor HPLC Assays for Rapid Process Development, In-Process Monitoring, and Validation of AAV Production and Purification. *Pharmaceutics* 2021; 13(1), 113.

63. Gimpel AL, Katsikis G, Sha S *et al.* Analytical methods for process and product characterization of recombinant adeno-associated virus-based gene therapies. *Mol. Ther. Methods Clin. Dev.* 2021; 20, 740–754.

64. Thakur G, Bansode V, Rathore AS. Continuous manufacturing of monoclonal antibodies: Automated downstream control strategy for dynamic handling of titer variations. *J. Chromatogr. A.* 2022; 1682, 463496.

65. Rüdt M, Brestrich N, Rolinger L, Hubbuch J. Real-time monitoring and control of the load phase of a protein A capture step. *Biotechnol. Bioeng.* 2017; 114(2), 368–373.

66. Thakur G, Hebbi V, Rathore AS. An NIR-based PAT approach for real-time control of loading in Protein A chromatography in continuous manufacturing of monoclonal antibodies. *Biotechnol. Bioeng.* 2020; 117(3), 673–686.

67. Iglesias Jr CF, Ristovski M, Bolic M, Cuperlovic-Culf M. rAAV Manufacturing: The Challenges of Soft Sensing during Upstream Processing. *Bioeng.* 2023; 10(2), 229.

 DOI: 10.18609/cgti.2023.082

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Thakur G, Mink S, Bak H and Tustian AD are employees of Regeneron Pharmaceuticals Inc. and have stocks/stock options within the company.

Funding declaration: Thakur G, Mink S, Bak H and Tustian AD received financial support for the research, authorship and/or publication of this article from Regeneron Pharmaceuticals Inc.

ARTICLE & COPYRIGHT INFORMATION

Article source: Invited.

Revised manuscript received: May 22 2023; **Publication date:** June 2 2023

CELL & GENE THERAPY INSIGHTS

VECTOR BIOPROCESSING & RAW MATERIALS

SPOTLIGHT

Setting up a one-stop shop: bringing viral vector manufacturing in-house

Danielle Steele
Moffitt Cancer Center

"...developers will have a greater choice of facilities with which to work. There will be more opportunities for collaboration and specialization..."

VIEWPOINT

On March 30th 2023, David McCall, Senior Editor, BioInsights, talked to Danielle Steele, Manager, Viral Vector Production at the Moffitt Cancer Center, about current trends and key considerations in viral vector manufacture. This Viewpoint article is based on that interview.

Cell & Gene Therapy Insights 2023; 9(3), 311–314

DOI: 10.18609/cgti.2023.049

IN-HOUSE VERSUS OUTSOURCED VIRAL VECTOR MANUFACTURING

Currently, many viral vector manufacturers are choosing to bring plasmid manufacturing in-house to reduce dependency on others for critical raw materials – a capability that becomes increasingly important as manufacturers plan expansions in capacity. Our new facility at Moffitt Cancer Center is a great example of an organization making the choice to bring vector manufacturing capabilities in-house and start its own small manufacturing facility within the existing Cell Therapies Core.

The Cell Therapies Core is a services core for the institution, which manufactures both standard-of-care and experimental cellular products in support of investigator-initiated trials and industry collaborations. However, until now, the clinical investigators at Moffitt have outsourced vector production for early-phase trials. Setting up the vector production facility for Moffitt makes us a one-stop shop, meaning that investigators can both have their vector made and have transductions and patient care work performed here. I was brought in to manage this viral vector production project alongside the experienced Cell Therapies team.

The current trend of CDMO expansion within the advanced therapies space means that developers will have a greater choice of facilities with which to work. There will be more opportunities for collaboration and specialization, and the possibility to form partnerships to guarantee a pipeline of work for a given facility. From a facility standpoint, that could be a double-edged sword: it can be useful to know that you have work in the queue but equally, it can be risky to only depend on a single industry partner. As a facility manager, I want to strike the balance between serving Moffitt's investigators first and foremost, but also cultivating relationships to diversify our service pipeline.

When a pharma or biotech company or academic institution decides to bring facilities in-house, there is a ripple effect. If more facilities make vector, then more facilities will need to certify vector, and more people will use the already overworked testing labs, adding to the backlog. The testing labs in this space will continue to see increased demand. Understandably, they are going to put their resources into the biggest accounts, so for example, big pharma accounts may receive better customer service than the newer, smaller facilities. It might be a good opportunity for testing labs to specialize in their project management by putting project managers onto the smaller accounts in order to nourish them.

FROM CINCINNATI CHILDREN'S TO MOFFITT: A TALE OF TWO VECTOR MANUFACTURING FACILITIES

I came from Cincinnati Children's Hospital, which has strong parallel cell and vector manufacturing groups. The vector facility in Cincinnati has three clean room suites and 10–12 technicians on staff at any given time. The hospital's research viral vector core makes products both for investigators based there and for others at the University of Cincinnati. They can perform pilot and feasibility studies for GMP clients and produce large-scale retroviral vector batches of ~30 L, in addition to 60 L GMP lentiviral vector batches. They have dedicated personnel to manage certification testing, product storage and inventory, and shipping.

Moffitt Cancer Center also has an experienced Cell Therapies group and has much of the required infrastructure in place already, which we can leverage as we develop viral vector production capacity. Moffitt already has a Facilities and Operations department that takes care of environmental monitoring and receipt of raw materials. We have a Laboratory Information Management System (LIMS) and an electronic quality management system, so we are adding capabilities to an established facility. For the new vector production space, we have chosen a modular clean

DOI: 10.18609/cgti.2023.049

room facility, with trailers custom-designed for vector production and support/storage. As the facility ramps up, we will tailor our focus to what Moffitt's investigators have asked for, which is small-scale production of mainly gammaretroviral vectors to start.

Many of our partners want to work with stable producer lines, and we are eager to move into bioreactor work, but finding a GMP-friendly fixed bed bioreactor that works for our scale was a challenge. Reminiscent of Goldilocks and the Three Bears, out of the three systems we looked at, one manufacturer's system was too large for us, and another manufacturer's was too small. The 'just right' fit for us was a system that has six possible vessel configurations and so six different surface area capacities available for runs. We like the fact that this will let us offer our clients a range of product scales, from small proof-of-concept work to larger pilot runs. Similar challenges were faced in the selection of the tangential flow filtration (TFF) system; we found ourselves in the 'grey area' between two system sizes, and needed to select the system that best let us work at both ends of our production capacity.

Another challenge is selecting and sourcing cell lines and packaging plasmids with the intellectual property provisions to let us work with both academic and industrial collaborators. The size and scope of our facility are also influencing the order in which we develop our services and roll them out. As our investigators have shown interest in stable producer cell lines, we will be starting with those in the iCELLis. We plan to grow those adherent cells and move into transient transfection, to build out those services in a logical order.

KEY PROCESS- & RAW MATERIALS-RELATED CONSIDERATIONS THROUGH DEVELOPMENT

Both raw material- and process-related considerations have been greatly influenced by the sector's experience of the COVID-19 pandemic. On the process side, both certification testing labs and shipping issues were causing delays. White glove couriers were having difficulties even in obtaining dry ice. During the pandemic, couriers tended to subcontract with local, less experienced couriers on the ground, and communication suffered as a consequence. There is supposed to be a seamless chain of operations protecting a critical product, but during COVID, there were some places where that chain became weak. The lesson to take is that selecting and building a relationship with a trusted courier is not something to be overlooked. You have made your product with great care; once it is in someone else's hands, you need to ensure they are completely trustworthy.

In terms of raw materials, unless you work in the world's most well-suited facility, you need to find a balance between ensuring you have enough raw materials for manufacture, and enough storage space to physically store them in a controlled/defined environment. This is especially critical with large items such as CellSTACKS®. At Cincinnati Children's Hospital, this was a challenge for us; we found the limits of our controlled storage space quickly when we needed to stock up on key items. For a facility that uses material at a steady rate, it is possible to take advantage of 'deduct and hold' arrangements with vendors. For a smaller facility, you are not going to have that purchasing power, so active management of critical items is even more important. However, the smaller your facility is, the less likely you are to have industry-scale formalized inventory control systems.

In addition, you can never be too careful with the critical scrutiny of every single aspect of a raw material – from the receipt, through the documentation review, to the point of use. A material can come in with the label and with the Certificate of Analysis you are expecting, but at the point of use, you could realize that the manufacturer has put the wrong product in that container. The bottom line is: trust no one and question everything.

It is important to train your staff to embrace a culture of questioning in order to protect your product and facility.

LOOKING TO THE FUTURE OF VIRAL VECTOR MANUFACTURE

The shift from static culture to bioreactor culture is huge. This gives us the ability to actively monitor our cultures' dissolved oxygen and pH, providing data points that we can respond to with a pre-programmed control loop. It is also increasing the standardization and the homogeneity of processes. Greater control leads to greater optimization, increased yields, and increased efficiency.

This shift also opens up a space in the market for quick, easy, informal diagnostic tools, such as the Lenti-X™ GoStix™ from Takara Bio. These are lateral flow assays for quantitative analysis of p24. Similarly, Unchained Labs is making many plug-and-play devices for lenti vectors, including one that can perform empty and full analysis, p24, and titering assays in a single unit the size of a printer. For the future, I would like to see similar products coming through for retro vectors.

BIOGRAPHY

DANIELLE STEELE is a graduate of Miami University (Ohio) and the University of Florida. Danielle formerly supervised laboratory operations and quality control at Cincinnati Children's Hospital's Vector Production Facility. She has also managed lab services for domestic and global clinical trials at Medpace Reference Laboratories. Now at Moffitt Cancer Center in Tampa, Florida, Danielle is Manager of Moffitt's new Gene Engineering Facility for viral vector production, opening in summer 2023.

AFFILIATION

Danielle Steele
Manager, Viral Vector Production at the Moffitt Cancer Center

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given his approval for this version to be published.

Acknowledgements: None.

Disclosures and potential conflicts of interest: The author has no conflicts of interest.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

Article source: Invited.

Revised manuscript received: Apr 5 2023; **Publication date:** Apr 11 2023.

DOI: 10.18609/cgti.2023.049

CELL & GENE THERAPY INSIGHTS

Analytics Channel

CELL & GENE THERAPY INSIGHTS

ANALYTIC CHANNEL

April 2023
Volume 9, Issue 3

INTERVIEW

Key considerations & development of regulatory guidance in CMC

David Ordóñez del Valle

CELL & GENE THERAPY INSIGHTS

ANALYTIC CHANNEL

CHANNEL CONTENT

INTERVIEW

Key considerations & development of regulatory guidance in CMC

David McCall, Senior Editor, BioInsights, speaks to (pictured) **David Ordóñez del Valle,** a Quality Assessor in the Biological Products Division of the Agencia Española de Medicamentos y Productos Sanitarios (AEMPS)

Cell & Gene Therapy Insights 2022; 9(3), 327–333

DOI: 10.18609/cgti.2023.051

Q What are you working on right now?

DODV: I am currently a Quality Assessor in the Spanish regulatory agency, the Agencia Española de Medicamentos y Productos Sanitarios (AEMPS). One of my main areas of focus is the evaluation of clinical trial applications in which the investigational medicinal product (IMP) is a biological medicine, so I work with products such as monoclonal antibodies and cell and gene therapies. We participate in some Scientific Advice with both academia and companies, and we also engage with industry regarding the central European Medicines Agency (EMA) procedures for granting marketing authorization. In addition, we are now working on the new clinical trial application portal called the Clinical Trial Information

System (CTIS), which on the 31st of January of this year became the only method by which sponsors can submit their trial applications moving forward.

Q What for you have been the key European regulatory guidance developments in the CMC area for the cellular immunotherapy field over recent times, and why?

DODV: In my opinion, the development of clinically successful chimeric antigen receptor (CAR)-T cell-based therapies has provided the opportunity for substantial development within regulatory bodies. The manufacture of these products includes a combination of various different processes and steps, including cell isolation, vector production, and effector cell manufacture. These different processes raised several challenges, which required updates to guidance from the regulatory point of view. Moreover, this field and its' regulatory environment are still undergoing constant evolution. For example, today, we must consider not only the processing of the vector and cells, but the genome editing of these cells to improve their quality and biological activity/potency. When this technology is implemented in the clinical setting, we as regulatory assessors will need to be prepared to provide an appropriate evaluation. In this regard, a recent update of the guideline on genetically-modified cells has been released (EMA/CAT/GTWP/671639/2008 rev. 1 corr [1]) which includes not only guidance for chemistry, manufacturing and control (CMC) but also for both non-clinical and clinical aspects. Additionally, the development of GMP guidance specific to ATMPs [2] introduced the required flexibility for the manufacturing and control of this type of product.

Q Your area of expertise also incorporates RNA-based cancer immunotherapies. What have been some important developments in that particular field?

DODV: The current mRNA-based COVID-19 vaccines opened the field and the regulatory point of view for the development of novel RNA-based therapeutics. RNA-based cancer immunotherapy development started a long time ago now, but those products did not really enter the regulatory space. The experience gained through the qualification of the COVID-19 vaccines will be applicable to new RNA-based therapies, but it is important to remark that even though those vaccines were thoroughly researched, their market authorizations were performed in the context of an emergency situation and so followed an accelerated pathway. Novel RNA-based therapeutics, on the other hand, will need to be evaluated via the current standard evaluation pathway. That said, there is some specific

DOI: 10.18609/cgti.2023.051

guidance for RNA therapies under development in the EU, which I hope will be available soon for public consultation.

Q As a regulator who focuses on quality/CMC, can you share any common pitfalls or issues that you see sponsors falling foul of in this area? And do you have any related advice for how to avoid them?

DODV: The dossiers we receive for clinical trial applications or marketing authorizations are usually of good quality. However, there are some common mistakes we find when we receive these dossiers. The first relates to good manufacturing practice (GMP) issues. This is a CMC/quality topic for Inspectors, but we as Quality Assessors must evaluate with regards to the GMP/qualified person (QP) certificates. When the sponsor gives us the list of GMP certificates for every manufacturing or testing site they plan to use, we usually find that there are some missing certificates, or that some certificates have expired. This issue is easily resolved by obtaining a new certificate, but nonetheless, this results in delays to the authorization of the clinical trial, or more importantly, to the final marketing authorization of the product. It is important to acknowledge that this is not solely a sponsor problem – due to the COVID-19 pandemic, all regulator GMP inspections were delayed.

Another key thing to bear in mind is the high importance of information relating to comparability exercises conducted during development. We usually ask sponsors to summarize this information because the marketing authorization applications are generally the result of years of development of a product, involving several changes of manufacturing and/or testing sites. The length and complexity of this development process can often result in some information being lost. My main advice would be to summarize the comparability information as much as possible whilst also ensuring that it is complete.

Lastly, I often see issues associated with acceptance limits. This is a constant problem with dossiers. We frequently need to ask the sponsor to tighten acceptable limits in the drug substance or drug product specifications. This is most evident where a sponsor has manufactured several clinical batches but continues to maintain the acceptable limits for the development batches. An unwritten rule is to apply a mean ± 3 standard deviation to the acceptable limit for quantitative tests, but the acceptable ranges should reflect manufacturing experience and manufacturing capabilities. For example, in one of my evaluations there was an impurity which was consistently determined to be below 0.1 ng/mg of the product. However, in one particular batch the Sponsor showed this impurity to be 4 ng/mg. This result complied with the original specifications of the impurity because they were set at less than or equal to 10 ng/mg, but it was completely different from the rest of the batches, and this deviation was not evaluated. Something clearly happened during the manufacture and/or purification of the product, but we were unable to ascertain what that was from the data supplied.

"... product development can take years, and it is quite reasonable to update analytical methods and technologies accordingly, as you progress from early- to late-stage development."

Q What are the key regulatory considerations for, and repercussions of, analytical technology changes during development – e.g. in terms of comparability?

DODV: As I mentioned previously, product development can take years, and it is quite reasonable to update analytical methods and technologies accordingly, as you progress from early- to late-stage development. From a regulatory point of view, we consider that companies will apply the state-of-the-art technologies in product characterization and control at any given time, and stay within the bounds of this methodology for all their batches, wherever possible. That would be the optimal scenario, but we know that some batches may suffer degradation during storage and thus, would not be applicable to determine the result with a new technology. In any case, if the company can evaluate both the former technology and the new technology in a way that demonstrates they are comparable, it will be acceptable.

Q Which analytical tools and methodologies can help meet regulatory expectations in terms of enabling characterization of immunotherapy products that involve genome editing?

DODV: At this point in time, our agency has limited experience in the assessment of genome edited products. What I can say is that the safety of these products would be the primary consideration when assessing product quality at the moment. Of course, gene engineering tools such as long and short RNA molecules, recombinant proteins, and viral vectors, are usually evaluated in quality dossiers, so we know that type of product very well. But genome editing is still such a new field in the therapeutic context that the first area of concern must be safety.

In terms of characterizing a gene edited product, it would be desirable that the proposed effect – for example, lack of expression of T cell receptor in CAR-T cells, which is a typical example of this type of genome editing – is easily and reproducibly determined.

DOI: 10.18609/cgti.2023.051

"...it would be important to determine whether a genome editing procedure involves an *ex vivo* or an *in vivo* strategy. It could be considered that an *ex vivo* approach is better in terms of safety, but we are aware that there might be situations which will require the *in vivo* approach."

Furthermore, in an optimal scenario, this effect should not be reversed. Regarding potency, it would be important to determine that the genome editing improves the product's biological activity. Last but not least, it is critical to determine and avoid off-target editing sites. The detection of off-target editing sites is currently the main safety concern about this kind of product and requires a very precise characterization.

I would also like to add that it would be important to determine whether a genome editing procedure involves an *ex vivo* or an *in vivo* strategy. It could be considered that an *ex vivo* approach is better in terms of safety, but we are aware that there might be situations which will require the *in vivo* approach.

Q What are your thoughts on how to manage potency assay development in an accelerated clinical development timeframe? For instance, is a potency assay matrix the best path to follow from the early stages of development?

DODV: There is a draft guideline in the EU for investigational ATMPs (EMA/CAT/852602/2018 [3]) that considers that a product in the early stages of development only needs a single reliable, qualified method to determine biological activity before the first phase of a clinical trial – so in other words, when you are planning for a first clinical trial, you need just one potency/biological activity test to be developed. We do consider that developers may wish to accelerate the potency assay development timeline, and so a potency assay matrix would also be a reasonable option at this stage from the regulator's perspective. Of course, the key consideration here for companies looking at establishing a potency assay matrix in preclinical development would be the potential delay to their Phase 1 clinical trial application. In conclusion, this would be a question for the regulator to answer on a case-by-case basis. Therefore, companies should consider contacting their specific regulatory body directly to discuss their specific requirements and pathway for potency assay development, whether it be their national regulatory body or in the case of the European Union, the EMA.

Q On that topic, do you have any additional advice to cell and gene therapy developers in terms of how to maximize the value of the CMC-related help available from regulators today?

DODV: Here in Spain, we provide a lot of regulatory support for academia and companies, most frequently at the preclinical stage in preparation for a clinical trial application. This can be through informal discussion meetings or via formal Scientific Advice procedures. In this line, our Agency has an Innovation Office (innov_spain@aemps.es) that will help the Sponsors with the steps to follow to set a meeting. The situation we most often encounter is a Sponsor that wants to start a clinical trial, but does not have the background to prepare a clinical trial dossier for a cell or gene therapy product. This may also occur when Sponsors are not familiar with the procedure in the EU. In both cases, Sponsors should prepare a clear presentation of what they want to do, focusing on the design of the manufacturing process and definition of the product specifications. If these two topics are clear following a meeting with the regulatory body, it will help to accelerate their development. Another important point at this stage of development is product safety, of course. The sponsor must prepare a clear strategy to avoid viral and non-viral contaminants, and the regulatory bodies can help them understand how that can be achieved.

Q Finally, are there any likely or expected regulatory developments coming through in the foreseeable future that will impact the cellular immunotherapy and RNA-based cancer immunotherapy spaces, and how should developers and manufacturers prepare for them?

DODV: A draft of the modified European pharmaceutical legislation will be released soon for public consultation. It will take some time until this legislation come into effect, but this will be a significant step to update the current regulatory framework and adapt it to the new therapeutic approaches that have emerged in recent years.

BIOGRAPHY

DAVID ORDÓÑEZ DEL VALLE is a Quality Assessor at the Spanish Agency for Medicines and Medical Devices, Biological Products, Advanced Therapies and Biotechnology Division. He obtained a Degree in Biology by the Universidad de Oviedo (Spain) and specialization in Immunology at Hospital Puerta de Hierro-Majadahonda (Madrid, Spain). Dr Ordóñez del Valle obtained a PhD at Universidad Complutense de Madrid (Spain). After 2 postdoctoral experiences, first at Medizinische Hochshule Hannover (Germany) and later at Massachusetts General

DOI: 10.18609/cgti.2023.051

Hospital (Boston, MA United States), he then returned to Spain and moved his interests into the Regulatory environment.

AFFILIATION

David Ordóñez del Valle
Quality Assessor in the Biological Products Division of the Agencia Española de Medicamentos y Productos Sanitarios (AEMPS)

REFERENCES

1. European Medicines Agency. Quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells - Scientific guideline.
2. European Commission. EudraLex The Rules Governing Medicinal Products in the European Union Volume 4 Good Manufacturing Practice. Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products.
3. European Medicines Agency. Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials - Scientific guideline.

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given his approval for this version to be published.
Acknowledgements: None.
Disclosure and potential conflicts of interest: The authors have no conflicts of interest.
Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: This article is based on an interview with David Ordóñez del Valle carried out on Feb 2 2023.
Interview held: Feb 2 2022; **Revised manuscript received:** Apr 3 2023. **Publication date:** Apr 14 2023

APRIL 2023 Volume 9, Issue 3

CELL & GENE THERAPY INSIGHTS

LATEST ARTICLES:

INTERVIEW

New directions and evolving challenges in the CAR-T cell therapy space

Roisin McGuigan, Editor, BioInsights, talks to **Amir Hefni**, Global Head, Cell & Gene Therapies, and **David Kuzan,** Global Medical Head, Cell & Gene Therapies, Novartis

AMIR HEFNI is the Global Head for Cell & Gene Therapy at Novartis. He currently leads the development and implementation of the Global C&G strategy across indications and disease areas. Amir joined Novartis from Ipsen, where he was Global Asset Head, Oncology and led the launch of multiple indications across the globe as well as drove a rich Life Cycle Management program. Previously, he was with Bristol-Myers Squibb (BMS) for 11 years, roles included Leading Thoracic indications for US Oncology (NSCLC, SCLC, and SCCHN). Furthermore, Amir brings a wealth of experience working across different countries including US, UK/Ireland and Sweden. Amir holds a BSc and PhD in Immuno-Pharmacology from King's College London, University of London (UK) and a MBA from Warwick Business School, University of Warwick (UK).

DAVID KUZAN is the Global Medical Head for Cell & Gene Therapy at Novartis accountable for development and execution of C&G medical strategy across global, region and country and execution of the medical affairs program for the C&G pipeline. David holds a Masters in Middle Eastern Politics, a Masters in Physiological Sciences, a medical degree from the University of Oxford and a Diploma in Pharmaceutical Medicine.

Cell & Gene Therapy Insights 2023; 9(3), 188–193

DOI: 10.18609/cgti.2023.027

Q Novartis has been engaged these past several months in a major reorganization and redirecting of activities in the cell and gene therapy space – can you run us through the key changes?

AH: Novartis has undertaken a significant transformation in order to build a more focused, 'pure-play' innovative medicines company with a clear focus on five core therapeutic areas as well as key technology platforms, including cell and gene therapies.

As you know, cell therapy is an advanced therapeutics platform, in a space where science is developing fast. That active landscape is volatile and fast-paced. As an organization, we need to ensure that we can be agile to drive agile innovation, which our new organizational structure now allows us to do.

DK: Cell and gene therapies can be a complex space, so reducing that complexity is key. Internally, we aim to become more agile, make decisions faster, and engage better, which is critical to our future success.

AH: Becoming more nimble and agile will make us more competitive and most importantly, allow us to accelerate innovative treatments to patients with high unmet need. We have an exciting pipeline both in terms of development as well as in discovery. With a simpler organization, you also have the advantage of faster resource allocation and faster decision-making to ultimately allow a faster cell therapy pipeline.

Q Can you give us some more detail on what the commercial and R&D portfolios will look like, both in the immediate and longer-term futures?

DK: In the immediate term, we have Kymriah®, the first – and currently, only – approved CAR-T product with indications in leukemia, large B-cell lymphoma (LBCL), and more recently, follicular lymphoma (FL). While we have pioneered cell therapies with the first approval, there is still a lot to learn; our current research focus

"..our current research focus with Kymriah is on understanding the factors that influence outcomes and how management can be optimized for the benefit of patients."

– David Kuzan

with Kymriah is on understanding the factors that influence outcomes and how management can be optimized for the benefit of patients.

We also have assets being developed on our next-generation, rapid platform, T-Charge. As a platform, T-Charge preserves the stemness of T cells – the ability for them to expand, persist, and mediate a prolonged immune attack. This should deliver more potent and durable products. In addition, since the manufacturing process time is less than 2 days, it gives the opportunity to improve accessibility, which is often an issue in this therapy area.

On the platform, we are especially excited about our lead program YTB323, a CD19-directed CAR-T for large B-cell lymphoma and follicular lymphoma, and PHE885, a B-cell maturation antigen-directed CAR-T in multiple myeloma. The current data is extremely encouraging. We presented Phase 1 data in the large B-cell lymphoma cohort a few months ago at the American Society of Hematology (ASH). The final products appear to include younger T-cell phenotypes compared to potentially exhausted effector subsets in traditional manufacturing with corresponding complete response (CR) rates of 73%. Response also seems to be extremely durable as at 6 months, the CR rate is over 60% at our recommended dose. We are now moving that forward into further development.

In the longer term, we also have several preclinical assets with the potential to leverage the same platform and expand into other malignancies and even non-oncology indications.

Q Stepping back for a moment, what's the view from the 'front line', so to speak – both at those hospitals and clinics already providing licensed CAR-T cell therapies to patients, and those seeking to bring them online? What are some of the key challenges they are facing, and how is Novartis seeking to alleviate them?

DK: The challenges have evolved over time. The challenges that we saw when we were starting out revolved around regulations, frameworks, and processes that had to be created. Kymriah is the first CAR-T therapy to ever be approved by the FDA, but we now have treatment pathways and processes that allow us to deliver at scale. We have also seen an evolution in terms of patient management and the safety profile of the therapy, reflecting the clinical and site expertise in delivering and handling a CAR-T product and patient. At the start, there were times when CAR-T was infused in the intensive care unit, whereas we now see routine outpatient administration in some centers.

The manufacturing challenges have also evolved. Meeting global demand in the commercial setting is a very different scenario to manufacturing for a clinical trial or in an academic setting, with capacity needs that shift from treating a relatively small number of patients with largely predictable recruitment to operating at a global scale with the challenges of predicting demand.

Since increasing our capacity, manufacturing has become much less of an issue. Currently, the challenges we face are those that we often see in other therapy areas, such as identifying

patients in a timely manner, considering the referral to a CAR-T physician, the treatment pathways, how to optimize outcomes, and how we can help expand access into new populations, new indications, and new geographies. We are always collaborating with and learning from medical experts to develop a better understanding of how we can help address these challenges.

AH: Over the past few years, Novartis has built up tremendous capability and infrastructure that allows us to deliver CAR-T therapy to healthcare practitioners (HCPs) and patients around the world with an extremely high level of reliability and manufacturing success.

Q What are the evolving challenges in clinical development – for example, in terms of the combination therapy development picture for cellular immunotherapies?

DK: Right now, in a real-world setting with Kymriah, an extremely important need is for a deeper understanding of the factors that influence outcomes. The challenge here is the large number of variables, including the T-cell fitness and function in the apheresis, the subsequent product characteristics, the disease characteristics, and variations in patient management. You mention combination therapies and again, there is a great deal to learn in terms of which combinations are advantageous. We are beginning to see systematic approaches to examine this. We also need to better understand which therapies can potentially impact T cells in a negative way where careful consideration of therapy sequencing is needed. Our T-Charge program is based on giving the T cells the best possible chance of success and we are focused on accelerating the assets on the T-Charge platform, through working with medical experts and health authorities.

Q Can you expand on the ongoing and future targets for the T-Charge platform, and other improvements that can ultimately improve patient access to these therapeutics?

AH: Our overarching objective is to advance the science with the aim of improving patient outcomes. The lead asset on our T-Charge platform is YTB323, a CD19-directed CAR-T. We are excited about the deep and durable efficacy that we are starting to see with YTB323, as well as lower rates of severe cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome adverse events.

The T-Charge platform's preservation of T-cell stemness is extremely important in terms of assuring greater proliferation and fewer exhausted T cells, i.e., a stronger immunological response to cancer. We have seen better results when using this platform with the efficacy data

DOI: 10.18609/cgti.2023.027

so far. This innovative platform allows the expansion of cells to take place *in vivo* rather than *ex vivo*, which is a huge advance in terms of increasing the effectiveness and reducing the adverse events profile.

Time to patient is critical, as these are sick patients who need treatment as quickly as possible. The new T-Charge platform manufactures YTB323 in 2 days or less. This is a significant change to the manufacturing process of CAR-Ts today. We are working to announce our door-to-door time over the coming weeks as we shift manufacturing in our clinical trials from our pilot site into commercial manufacturing for YTB323.

"The new T-Charge platform manufactures YTB323 in 2 days or less. This is a significant change to the manufacturing process of CAR-Ts today."

– Amir Hefni

We received some great feedback from HCPs at ASH and we look forward to announcing data updates over the coming months. With the T-Charge platform the door-to-door time can be greatly reduced.

DK: The data coming out of the T-Charge platform is all moving in the right direction. We are seeing that the stem-like phenotype is preserved and that it should drive potency and durability, which is being indicated in the clinical data. There is a lot of excitement internally and from our external medical experts.

Q What are the critical lessons that you draw from Kymriah's commercialization that will benefit the commercialization of future cell and gene therapy products?

AH: In my experience, having worked across multiple therapy areas, in different countries, and with varying degrees of complexity, cell therapy truly requires a unified one-team approach. The teams across discovery, development, manufacturing, commercialization, medical affairs, and access needs to work as one team. We place great importance on maintaining strong relationships and breaking down silos. Part of the Novartis transformation is to enable this, and for us to work as one cell therapy team across functions.

DK: CAR-T is a process that starts and ends with the patient, and the drug substance is the patient's T cells. This brings a great deal of complexity and variability in patient management, scheduling, and logistics. We often talk about medical expert engagement and insight-gathering, but we need to deeply understand the challenges that are faced by physicians and patients. It is vital to have that true understanding today and for the future.

Q What are the key current trends and potential disrupters in immuno-oncology therapeutic innovation? How will Novartis remain nimble enough to stay ahead in this fast-paced field?

AH: Cancer waits for no one and therefore, trying to target cancer using multiple modalities across the industry is critical. Anything that will benefit the patient is something that we would encourage and seek to learn from.

Internally at Novartis, we continue to look at multiple modalities in and outside of cell therapy. We are focusing our efforts on five therapeutic areas and three key technology platforms. Cell therapy is currently focused on the hematological area with Kymriah, our current CAR-T. Although today we have multiple CAR-Ts in DLBCL and follicular lymphoma, Kymriah still remains the only CAR-T in pediatric acute lymphoblastic leukemia, a critical patient population with no other treatment option.

At Novartis, we have an unwavering commitment to delivering to patients in need. We need to bring our pipeline to patients as quickly as possible. We believe we are onto something exciting, as do our customers, so we need to figure out how to bring YTB323 and other assets on the T-Charge platform to patients as quickly as possible.

Then, beyond hematology and oncology, where do we go? We are looking at areas like immunology, and there is more to come on this over the coming months. Having a relentless drive to improve patient outcomes motivates us to move things as fast as possible.

Q Finally, can you each sum up some important priorities and goals that you have for your work over the foreseeable future?

DK: One tremendous part of the Novartis organization is the Novartis Institute of Biomedical Research, or NIBR, which is dedicated to early research and discovery, and the T-Charge platform is an example of its innovation. In addition, we have many highly motivated teams working across the company and we regularly engage with the world's leading medical experts and academic institutions in the field of cellular therapy. An important priority is to ensure the close collaboration between internal and external experts so we can unlock the full potential of what we can all do together.

AH: First, we need to continue to deliver Kymriah to patients around the world with absolute commitment. Second, all our colleagues are committed to bringing YTB323 and PHE885 to patients as quickly as possible. Simplistically, those are our two key short-term priorities.

The area of cell therapy and advanced therapeutic platforms sparks incredible passion, engagement, and commitment from teams around the world, who seek to do everything they can to support patient access to incredible medicines like Kymriah and others. I want to send out

DOI: 10.18609/cgti.2023.027

a huge thanks to our teams around the world because it is inspiring to see the incredible effort and commitment.

AFFILIATIONS

Amir Hefni
Global Head for Cell & Gene Therapy, Novartis

David Kuzan
Global Medical Head for Cell & Gene Therapy, Novartis

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *The named author takes responsibility for the integrity of the work as a whole, and has given their approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The authors are Novartis employees and stockholders.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

Article source: This article is written based on an interview with Hefni A and Kuzan D, held on January 16 2023.

Interview conducted: Jan 16 2023; **Publication date:** Mar 6 2023.

INTERVIEW

A payment innovation framework to meet the urgent need in advanced therapies

David McCall, Senior Editor, BioInsights, speaks to (pictured)
Mark Trusheim, Strategic Director, NEWDIGS,
Tufts Medical Center

Cell & Gene Therapy Insights 2023; 9(3), 335–340

DOI: 10.18609/cgti.2023.052

Q What are you working on right now?

MT: At NEWDIGS, we are working on payment innovation for drugs at scale and creating evidence through that innovation – an often-unexplored extension. We are accomplishing this through our two multi-stakeholder consortia: Financing of Cures in the United States (FoCUS); and Learning Ecosystems Accelerator for Patient-Centered, Sustainable Innovation (LEAPS). FoCUS concentrates on understanding new payment approaches for durable cell and gene therapies, whereas LEAPS concentrates on advancing learning healthcare systems through real-world evidence platforms.

Payment innovation at scale requires a platform on which to build. Historically, we have not thought of payment processes as leading to learning that can help refine the way we treat

people in the clinic. We hope that our new Payment Innovation Framework [1] will enable a better payment approach to help payers cover and reimburse, enable patient access to advanced therapies, and deepen understanding of how to improve the ways in which we use these therapies over time.

Q How do you reflect upon the past few years in the cell and gene therapy space and in particular, on how approved cell and gene therapy products have fared in the commercial setting over this period?

MT: The last year has been particularly exciting; we are now seeing gene therapies and CAR-T cell therapy approvals in the cancer space steadily arriving.

During the COVID-19 pandemic there was a pause, both due to the pandemic itself and to regulators needing to grow their understanding of the specific evidence needed to evaluate these novel products, often for very rare conditions. Over the last 6–12 months, though, multiple CAR-T cell therapies have been approved, alongside therapies in the gene therapy space for hemophilia and beta thalassemia among others. Over the next year, it is likely we will see therapies being approved in other areas, like sickle cell disease, Duchenne muscular dystrophy, and some retinopathies. There may also be second and third products approved in some indications, such as hemophilia.

From an uptake and adoption standpoint, gene therapy is more of a steadily rising tide than a tsunami. We are seeing great acceptance so far, but there is still an obvious need for the education of patients, healthcare providers, and payers. It is a time of great learning for everyone to make this as streamlined as possible. It is necessary for patients to have the information they need to make educated decisions. This takes time with brand new products coming to market, especially for these entirely new modalities.

Q Can you tell us more about the NEWDIGS and FoCUS consortia and their activities – how did they get their start, and what specific issues did they set out to address?

MT: NEWDIGS began nearly 15 years ago at the Massachusetts Institute of Technology (MIT), where we gathered with payers, developers, and patients to improve the regulatory approval process in order to speed appropriate patient access in ways that met all stakeholders' needs. We called this continuous process of learning 'adaptive licensing', which is now known as 'adaptive pathways' in Europe. In the USA, we see its principles in the combination of the FDA Accelerated Approval program; the Regenerative Medicine Advanced Therapy (RMAT), Breakthrough Therapy and Fast Track designations; and its Real-World Evidence programs. Adaptive Licensing combines contingent approvals and continuing evidence development to refine and expand drug labels, particularly for precision medicines and therapies for patients with high unmet medical

DOI: 10.18609/cgti.2023.052

needs. In the last year, we have moved to Tufts Medical Center to get closer to patients, where the action is.

As we have continued to grow and evolve, we have found that our community has transitioned from focusing on the upstream development and regulatory approval processes to seeing a need for systems change in healthcare further downstream. Downstream innovation for patient access was not matching the phenomenal scientific and clinical development innovation that we have seen with these new products.

Without collaborative efforts among patients, providers, payers, developers, and regulators to ensure that access is possible, the science is not going to have the impact that we want. So, we run consortia bringing together around 100 people from 50+ organizations to work together over time through a series of 'Design Labs'. These are firstly aimed at better understanding the challenges that are impeding appropriate patient access to new therapies. The participants then design solutions for those challenges, pressure test them in subsequent design labs, and even begin implementation planning to execute within their own organizations. Occasionally, the consortium itself pilots such implementation projects.

We believe real systems change on a global scale happens not from demonstrations within a limited scope, but by all the consortium member stakeholders and the broader healthcare system adapting and adopting those innovations in their day-to-day operations. We have historically concentrated on durable cell and gene therapies, but have recently done work in the cardiovascular, autoimmune, and broader rare disease spaces, too. We are seeing these payment innovations spreading from what began as cell and gene therapy concerns to broader applications across many medicine types.

Q Can you go deeper on the recently released Payment Innovation Framework? What is it, and what are some of the key principles underpinning it?

MT: The Payment Innovation Framework comes from both FoCUS and LEAPS. We saw a need from both sides to bring together a joint effort of payers and developers to guide a systematic approach towards doing payment innovation and value-based contracting for the many transformative medicines which nonetheless have currently uncertain clinical efficacy evidence due to their novelty or the rarity of the conditions.

In September 2022, we gathered 15 leading US drug developers and national and regional payers to discuss principles that we should use throughout the healthcare system to allow these new payment innovations to become standard practice, thereby making access to medicines more streamlined. Through these discussions, both payers and developers identified four major challenges they felt needed to be addressed through payment innovation.

Firstly, the therapeutic risk that we often see with these new transformative therapies poses a challenge. While they are exciting in their promise, oftentimes the evidence behind these therapies is still relatively immature, so payers perceive a risk of not knowing how well they will work in their particular populations – an issue exacerbated by the fact that these are frequently high-cost therapies for rare conditions. In such circumstances, there is a desire to ensure that

patients see the value of these therapies. Value-based contracting is a key tool to help understand the therapeutic risk and manage it, allowing for varying payments to be made to developers that are directly tied to the benefits that treated patients receive.

The second challenge is the actuarial risk that payers experience, particularly in these rare conditions. Anything in a new payment innovation approach that can help smooth that volatility through different kinds of pooling or payment spreading over time can be useful. For example, subscription models are gaining some traction – a separate approach to value-based contracting, but another form of payment innovation needed in the marketplace today.

The third challenge is finding ways to execute these payment innovations efficiently, without adding phenomenal costs to the healthcare system. This is where having data platforms and more standard operating procedures for how we execute and implement these contracts becomes important going forward.

The final challenge is around evidence creation. Usually, we do not think of financial contracts between developers and payers, and payers and providers, as also generating clinical evidence. However, the more we move into value-based contracting, the more we see the opportunity for them to add to the totality of evidence about how well these therapies deliver value to patients.

Ultimately, the developers and payers came together to agree on five core principles of what payment innovation should try to attain:

FIVE CORE PRINCIPLES

- **Ensuring patient-centric and equitable access.** Payment innovation should ensure and advance patient-centric and equitable access for patients to these therapies. It is absolutely essential to ensure that patients have access in an equitable way, and in a way that centers on their needs, as well as those of payers and developers. Payment innovation should not increase, and even seek to reduce, healthcare disparities.
- **Connecting access and reimbursement to the benefits patients receive.** It is important to connect patient access with reimbursement tied to the benefits patients receive. To do this, the value received by patients must firstly be measured, and then the payment needs to correspond to this measurement. For durable cell and gene therapies, this may mean multiyear payment approaches to assure that benefits are sustained over several years. It is important to align incentives across all stakeholders, so that all may share in the financial benefits of the therapeutic payment innovation.
- **Reducing financial volatility.** Particularly for rare conditions, financial volatility is often experienced by payers – even as few as one or two high-priced therapies in a given quarter can be a financially challenging income statement event for a small payer. New financial payment innovations that spread risk across multiple payers or longer periods of time are important.
- **Operational efficiency.** To achieve efficiency in operations, collaboration among many stakeholders is required. Since each product launches separately, we currently administer these products one at a time with a different data collection system for each product and payer, which is a high-overhead approach. We must find ways for developers to cooperate within and across therapeutic areas, and for payers to cooperate with their healthcare providers to build better and more standardized data collection mechanisms to measure patient outcomes efficiently. We need consistency across the healthcare system, so that patients, payers, developers, and physicians have a reliable, efficient, and consistent experience. Reducing payment innovation administration costs whilst maintaining accountability and flexibility should be a key goal, as should simplifying contracts and adjudication processes.
- **System evolution in response to new learnings.** We want to design the system to learn over time as well as share best practices and core principles among all stakeholders. Wherever and whenever possible, everyone needs to agree to share and be somewhat transparent about how well these value-based contracts perform, particularly on the clinical benefits side.

DOI: 10.18609/cgti.2023.052

These five principles are important for everyone. We think this is a qualitative change in both the scope and the attitude of how payers and therapeutic developers in the USA are thinking about scaling up payment innovation in a way that can be operated efficiently. These value-based principles are multiyear concepts that will not only help provide patient access today, but also add to the learning in order to optimize patient access in the future. A final area to mention is the great need to develop patient-centric outcomes metrics that can be efficiently measured.

Q Can you expand upon the key outcomes or action points stemming from the Payment Innovation Framework?

MT: I would break down the key action areas, which correspond closely to the challenges and principles discussed above, into the following:

- A patient-centered framework: All stakeholders saw the importance of ensuring patients had access to new and transformative cell and gene therapies as rapidly and appropriately as possible.
- Collaboration in value-based contracting: Working with payers and developers within their organizations to explain what value-based contracting and payment innovation entails is key to ensuring all parties understand the best practices and standard approaches required to realize it.
- Developing functional standards for data collection: Standards must be based on patient-centric health outcomes and be able to gather information in a robust, reliable, and efficient way. Financial transactions between developers and payers must be able to rely upon these measured outcome metrics.
- Shared learning and best practices: A flexible and customizable infrastructure of concepts and capabilities shared amongst the entire healthcare system will decrease the need for individual organizations to learn and invent entire processes alone.
- Regulation innovation and government policy: Regulation changes are likely required to support the scale and the implementation of these new payment innovations. This includes removing regulations that are obstacles to flexibility.

REFERENCE

1. Payment Innovation Framework.

BIOGRAPHY

MARK TRUSHEIM is Strategic Director, NEWDIGS at Tufts Medical Center where he also co-leads the Financing and reimbursement of Cures in the US (FoCUS) Project; and a Visiting Scientist at the MIT Sloan School of Management. Through MIT he has also served as a Special Government Employee for the FDA's Office of the Commissioner. Mark's research focuses on the economics of biomedical innovation, especially precision financing for patient access, precision medicine, adaptive pathways, platform trials, biosimilars, and digital health advances. Mark is also President of Co-Bio Consulting, LLC. His career has spanned big data at Kenan

Systems, marketing at Searle Pharmaceuticals, eHealth as Vice President of Monsanto Health Solutions, genomics as President of Cereon Genomics, and policy as the President of the Massachusetts Biotechnology Council. He holds degrees in Chemistry from Stanford University and Management from MIT.

AFFILIATION

Mark Trusheim
Strategic Director, NEWDIGS at Tufts Medical Center

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given his approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author is President of Co-Bio Consulting LLC.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

Article source: Invited.

Interview held: Feb 19 2023; **Publication date:** Apr 19 2023.

DOI: 10.18609/cgti.2023.052

CELL & GENE THERAPY INSIGHTS

Funding the future: cell & gene therapy development

Boyds' **Dr Nick Meyers,** Vice President of Product Development
& Dr Neil Fish, Vice President of Business Development

VIEWPOINT

"Moving forward, the traditional funding routes will continue to exist, and we hope there may be more government-led funding available to help companies accelerate their innovations."

Cell & Gene Therapy Insights 2023; 9(3), 295–298

DOI: 10.18609/cgti.2023.046

In this viewpoint article, Dr Nick Meyers, Vice President of Product Development, and Dr Neil Fish, Vice President of Business Development at Boyds, discuss the availability of funding for cell and gene therapy product development, and how organizations can differentiate themselves in the market to attract investment.

Securing funding for novel technologies and cutting-edge developments in the biotech sector is currently a challenge. But the push to develop technologies to meet unmet medical need, particularly cell and gene therapies, is considerable, and there are still sources of investment available for the well-prepared.

Cell and gene therapy is arguably one of the most exciting and dynamic areas of research and development today. Whilst the global cell and gene therapy community is still on a steep learning curve, with every clinical study that is conducted, we learn something new about how this technology can be used.

An apt analogy to how cell and gene treatments may progress is the development of drugs to treat human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDs). The very first versions of these drugs had high pill burdens and unpleasant side effects, and it took many years before more advanced versions of these drugs were developed and used optimally. These new therapies have had a transformative effect and now allow people living with HIV to have a normal life expectancy.

By comparison, cell and gene therapy is currently at a much earlier stage, yet every day we are inching closer to where we ultimately need to be. The recent approval of the gene therapy product Adstiladrin for the treatment of non-muscle invasive bladder cancer was an exciting landmark for the industry and demonstrated that it is possible to effectively target more challenging cancers such as solid tumors.

SECURING FUNDING

Following two to three years of record investment, funding for the development of new products in the cell and gene therapy space in some geographic regions has slowed. Adverse market conditions caused by a range of factors, including rising interest rates, the continuing effects of Brexit and the ongoing war in Ukraine have all impacted investment.

By way of an example, for UK biotechs there are currently limited sources of funding available for cell and gene therapy development. Those with access to finance from investors are usually provided with it in discrete tranches and in a sequential manner, thereby slowing down development as activities cannot be conducted in parallel. This can lengthen development times whilst at the same time eating into the patent life of a new cell and gene therapy product.

Broadly speaking, however, there is still investment available for cell and gene therapy development – it's just much harder to get at the moment than it is during more normal economic conditions.

Funding for investment can be sourced from venture capitalists, specialist health tech and biotech investors, angel investors, the investment arms of pharma companies, pharma companies themselves, and research councils. There is also private equity and the stock market as sources of funding.

With some of the cell and gene therapies targeting rare diseases – especially ultra-rare diseases – we are also beginning to see patient groups, patients' families, and even crowdfunding coming into play. The disconnect here is that developing these types of therapies is extremely costly and additional funds will certainly be required as a development project progresses towards the market.

Nonetheless, funding is only going to be accessible to those companies that position themselves well and provide a compelling story and reasons to invest. Undeniably, these companies need strong initial data to convince investors they are worth backing. It is therefore key to have technology that is credible and has a well understood mechanism of action that captures people's imaginations.

Having robust forward development plans in place is a must, regardless of the current stage of development. Planning demonstrates to investors that the company has thought about and understands where the key inflection points will be; the points where the program will require further injections of

DOI: 10.18609/cgti.2023.046

funding, and the go/no-go data or decision points needed to trigger them.

There has also been a discernible shift in the maturity of financial investors in the sector since the late 2000s, with investors now truly understanding the science, development plans and whether timelines presented by companies are realistic or not. Of course, most potential investors will conduct some degree of due diligence, either in-house or using external expert consultants.

Demonstrating proof-of-mechanism preclinically in a relevant animal model used to be a significant milestone triggering substantial investment, although the weaknesses of some of the data from such studies and the fact that many animal models do not properly recapitulate the disease processes/pathology in patients means that increasingly now, and understandably, the funds required for later stage development and pivotal clinical trials are only unlocked when clinical proof-of-concept has been demonstrated.

Moving forward, the traditional funding routes will continue to exist, and we hope there may be more government-led funding available to help companies accelerate their innovations. The biotech industry is well known for having investment cycles and we hope that as inflation and interest rates fall, the biotech financing window will open again.

Many large pharma companies in Europe and prestigious research institutes in the US have set up their own foundations to make equity investments in young companies – and this may be something other countries look to replicate.

SCIENTIFIC & COMMERCIAL HORIZON SCANNING

Everyone now understands that with rare diseases, it is possible to substantially accelerate development timelines. This is because the extent of the toxicology package is often much-reduced compared with small molecule new chemical entities (NCEs), sample sizes in clinical trials can be much smaller, the number of clinical trials required may be fewer, and in some cases, conditional marketing approval whilst trials are ongoing is possible. The more rapid speed to market has made cell and gene therapies very attractive, but the duration of their efficacy and use is an important factor to consider. One of the key challenges for the industry is agreeing the cost of its therapies with payers vis-à-vis the duration of effect and long-term quality of life; for example, is the therapy curative resulting in a normal lifetime, and what happens if this breaks down and health deteriorates further over time, and/or additional treatment cycles are required?

Whilst a lot of seed-stage companies are spinouts from academia and based on excellent science, what investors are most interested in is how the product is received when it eventually comes to market. Sponsor companies must consider what the standard of care (SOC) is likely to be at that point, and what other drugs are being developed by competitors and are going to reach the market around the same time and/or have become SOC by that point.

Indeed, it is never too early to conduct some forward-looking scientific and commercial horizon scanning to understand how a new therapy can be positioned at the forefront of treatment at the point when it reaches the market. Something else that may help with this is, in our view, the consolidation of companies. By merging, biotech's may be able to offer a more attractive opportunity to investors and add critical mass and additional expertise to help balance out the development risk.

LOOKING TO THE FUTURE

There is a lot of hope and optimism in the area of cell and gene therapy. The clients we work with at Boyds are all trying their best to achieve amazing things, with some aiming to cure – not just treat – cancer, restore sight or hearing, and treat hitherto

intractable neurodegenerative diseases and other rare diseases.

Over the coming years, we're going to see more and more cell and gene therapy approvals across the US and Europe and more sophisticated technology, representing truly personalized medicine. Whilst personalized medicine has been talked about for more than 20 years without ever truly coming to pass, the power of cell and gene therapies means it is now becoming a reality.

BIOGRAPHIES

DR NICK MEYERS is Vice President of Product Development at Boyds. He is an experienced program director and project leader, with over 25 years' experience within the industry, working in large pharma, biotech and the CRO sector, delivering internally resourced and outsourced projects involving multi-disciplinary teams spanning different companies and geographies. Nick has a PhD in bacterial genetics from the University of Cranfield.

DR NEIL FISH is Vice President of Business Development at Boyds. With over 35 years' experience in the life sciences industry, Neil is a highly creative business development expert with an outstanding track record of completing deals. He has a BSc in Biological Sciences from the University of East Anglia and a PhD in Cell and Molecular Biology from Imperial College London.

AFFILIATIONS

Dr Nick Meyers
Vice President of Product Development, Boyds

Dr Neil Fish
Vice President of Business Development, Boyds

For further information, please contact: Sue Carr on 07809 727533, email: sue.carr@boydconsultants.com

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: Meyers N discloses he is an employee of Boyd Consultants Ltd and has a small share holding in the company. The other author has no conflicts of interest.

Funding declaration: The authors received no financial support for the research, authorship and/or publication of this article.

Article source: Invited.

Revised manuscript received: Mar 24 2023; **Publication date:** Mar 28 2023

DOI: 10.18609/cgti.2023.046

EXPERT ROUNDTABLE

Accelerating cancer therapies development: the importance of platform, process, and partnership

Charlotte Barker, CEO of BioInsights, speaks to **William Swaney,** President of Manufacturing, Expression Therapeutics, **John (Yoshi) Shyu,** Director, Global Scientific Applications, Corning Life Sciences, and **Nikhil Tyagi,** Director of Cell Therapy Process Development, Center for Breakthrough Medicines

WILLIAM SWANEY is the President of Expression Manufacturing LLC. He recently oversaw the buildout of Expression's in-house manufacturing cell and gene therapy manufacturing facility. He has three decades of experience in biopharmaceutical manufacturing of cell and viral vector products for human clinical trial use and has successfully overseen the manufacture of more than 70 GMP runs for both academic and commercial clients. His professional focus is the development of improved & scalable manufacturing platforms for different viral vectors and the manufacture of individualized patient-specific products for the treatment of cancer.

JOHN (YOSHI) SHYU is Director, Global Scientific Applications & Technical Support at Corning Life Sciences. Dr. Shyu and his team support product development, application, and market adoption, especially in the fields of bioprocessing, cell and regenerative therapy, viral vector/vaccine production, and 3D cell culture. Since joining Corning Life Sciences in 2008, Dr. Shyu has focused on helping customers overcome a broad range of challenges in research, development, and production, with particular emphasis on scale-up/out for cell and gene therapy and vaccine production, and advanced 3D cell culture.

NIKHIL TYAGI is the Director of Cell Therapy Process Development at the Center for Breakthrough Medicines in Pennsylvania, responsible for overseeing all cell therapy programs and initiatives. He has extensive experience in the field of cancer biology and cell therapy, has published numerous articles in leading scientific journals, and is known for his ability to bring together multidisciplinary teams to drive breakthrough innovations. Under his leadership, the Center for Breakthrough Medicines has become a trusted partner for clients looking to develop their cell therapy products, providing expert guidance and support throughout the entire process.

Cell & Gene Therapy Insights 2023; 9(3), 343–351

DOI: 10.18609/cgti.2023.054

Q What are the biggest challenges that the cell and gene therapy industry faces in getting products to market quickly?

WS: Cell and gene therapy companies, regardless of their development stage, all have the same goal of reaching the market. To get to market, you need to manufacture the product and complete initial early-phase clinical studies. Considerations will be different for the company based on its development stage. Drivers often include the disease indication, whether it is single or multiple treatments, and the platform chosen. The dosage and route of administration will also come into that discussion. In addition, it is worth noting the regulatory considerations as well as the intended markets you want to serve. Regardless of company size, everybody wants to build manufacturing facilities that are flexible, modular, and sustainable.

A big challenge currently facing early-stage startups is that over the last few years, venture capital funding and mergers and acquisitions have slowed down considerably. Many firms are cash-starved and are unable to capitalize on their ideas and move into the clinic. One of the big paradigms for early-stage companies is whether they outsource to a CDMO. If you are fortunate enough to be an early clinical-stage company with in-house manufacturing, talent acquisition, and retention will be key to getting products translated and into the market quickly. Supply chain management is also going to come into that, as well as regulatory compliance.

For commercial-stage companies, the lack of harmonization across some of the major global markets impedes moving things into the market. For example, requirements are different in Europe and the USA.

NT: As all cell and gene therapies are new, we do not have much data available on safety and efficacy. Over time, more data will become available and once we have an increased understanding of safety and efficacy, we should be able to bring more therapies to the market.

JYS: After seeing how the market has exploded in the last several years for cell and gene therapy, a common question is how do we get things faster? How do we get the product in an amount that is more relevant to the indications that scientists are studying? Having the right infrastructure, the right platforms, and the right support structure to generate enough material to be able to complete a program are all key considerations. One common challenge in the market is that despite our great ideas, we need to further understand what is needed from a workflow perspective to be able to accomplish our goals.

> "One common challenge in the market is that ... we need to further understand what is needed from a workflow perspective to be able to accomplish our goals."
>
> **- John (Yoshi) Shyu**

Q In addition to reducing time to market, how can we make cell and gene therapies more accessible?

NT: Cell and gene therapies are a promising field, and we have seen early results in diseases including cancer. The only problem is that these therapies are very expensive. Current cell therapies range from $300–500K per dose, and gene therapies are over $1 million per treatment. This is inaccessible to most patients.

If we can reduce the cost, then we can bring those therapies to the wider population. The main problem is how to do so. In my opinion, we must develop more cost-effective and affordable manufacturing processes. Can we reduce the length of the manufacturing process? Can we bring automation to these processes?

Allogeneic cell products could significantly reduce cost compared to autologous therapies and we would be less likely to experience supply and demand issues if bulk manufacture became possible.

Another consideration is the reimbursement landscape. Currently, our insurance policies do not cover these expensive cell therapies. We need to create more therapies with affordable manufacturing processes and work with governments to make these accessible to every patient.

WS: When these products come into the early phase of development, there are often still open manipulations or unclosed steps. This leads to requirements for working in the appropriate environment, for example in an ISO Class 7 room with ISO Class 5 biosafety cabinets in the USA, or with Grade A operations occurring in a Grade C room in the EU. If you can close those processes, you may be able to decrease the amount of clean room required, which would have a significant impact on the cost of manufacturing.

NT: Most cell and gene therapies are being developed in academic institutions and startups. Those companies and institutions have narrow pipelines, only working on one

or two products. They are making their own manufacturing facilities, which is adding to costs. If we can bring those manufacturing processes to CDMOs or large pharmaceutical companies that already have manufacturing facilities, we can reduce the risk of investing in a single-product pipeline and enable the production of more doses in a shorter timeframe.

> "If we can develop a more automated process – closed, and GMP compliant – we can reduce the length of the process and cut the cost of manufacturing."
>
> – **Nikhil Tyagi**

Q What are the key factors you would advise looking for when selecting a manufacturing platform?

JYS: One of the most important questions is: what is the life cycle of your process? If your product needs to be produced for the next year or two, does your platform allow you to produce two, three, or even five times the amounts you need right now? If so, your process will cover your life cycle for the next couple of years. If not, you may need to look at more nimble platform alternatives to increase size. The platform you are selecting needs to meet your current needs in addition to your future needs for the next several years, or the entirety of the process life cycle.

NT: As mentioned earlier, most of the manufacturing platforms for cell and gene therapies are currently open and manual. We need to develop more simple, affordable manufacturing platforms, and investigate both unit automation and end-to-end automation. Current cell therapies are very complex. If we can develop a more automated process – closed, and GMP compliant – we can reduce the length of the process and cut the cost of manufacturing. The first requirement is to develop an easy-to-use, affordable, robust, and reliable manufacturing platform.

For all cell therapies, we are dependent on source material from the patient, which shows large patient-to-patient variability. This underscores how important it is to develop a robust and reliable manufacturing platform to reduce manufacturing failure.

WS: We need to move towards flexibility, scalability, and simplicity in closing and automating processes. We also need the flexibility to do small-dose manufacturing, as there is still a huge unmet medical need for ultra-rare diseases, as well as produce larger product doses. The platform needs to drive both large and small requirements.

Q What are your thoughts on transitioning between platforms as you scale up and advance through the preclinical and clinical pipeline? And what are solutions providers like Corning doing to optimize that compatibility?

DOI: 10.18609/cgti.2023.054

NT: It is important to choose wisely when selecting a platform. Most of these therapies are being developed in startups and academic institutions that do not have a vision for Phase 3 and commercial manufacturing. Transitioning from preclinical to clinical becomes challenging, particularly in the case of cell therapies, which are not the same as small molecules or monoclonal antibodies. A small change, such as a change in the growth factor in the media, can result in a different product.

There is a great deal of demand from people who have already developed their process using standard, open, and flatware culture systems, but when moving from preclinical to Phase 1 or 2 need to improve their process. Service providers like Corning are helping us here.

Taking the example of an open stem cell therapy process, there is a scale-up limit on growing cells in T flasks or HYPERstack® cell culture vessels. Scale-out is limited due to the labor-intensive processes required. Platforms like flatbed bioreactors, fixed bed bioreactors, and stirred tank bioreactors are possible options, though comparability needs to be considered. If the process is developed in a 2D platform, it is challenging to move from 2D to 3D, as it will change the biological properties of your product.

I am currently working with Corning on the Ascent® FBR System which is a highly compatible platform. We can easily scale up the process, and close and automate steps. Companies like Corning and other service providers are working towards this, and as CDMO service providers, we are quickly adapting those technologies.

JYS: Transitioning between platforms at any stage of the manufacturing process is a pain point. If you are transitioning between platforms directly, the optimization time tends to be short, at a maximum of 2 months. When transitioning, you must ensure you have the correct support structure both from the supplier and the technical personnel to allow you to transition collaboratively. Technical assistance becomes a critical component for anyone transitioning between platforms.

WS: There are many learnings to be had at the early stage of preclinical development. We are on board with the concept of maintaining platform fidelity throughout the clinical development program. If possible, you should avoid switching platforms or producer cells, because that will affect some of the key quality attributes of your products. For example, if you went from using an adherent cell line to make lentivirus to a suspension cell line, this changes the downstream purification platform and may require expensive bridging studies to show comparability.

We are also working on the Ascent fixed bed bioreactor for that same reason. The data provides us with the ability to stay with our adherent cell lines, our plasmids, and our existing process. It is good to hear that other people are seeing the same benefits that we are seeing.

Q In what specific areas have you seen the greatest impacts of global supply chain shortages and price increases? What is your advice to others on how to address this ongoing issue?

"When we first resumed activities in our manufacturing facility, we were seeing lead times approaching 40–50 weeks for a customized product, even for simple things such as longer tubing or different connectors. We had to evaluate whether customized products were worth it..."

- William Swaney

WS: Early in the pandemic, there were huge supply chain issues with the acquisition of PPE and plastic labware. There was an inability to acquire multi-tray stacks and culture media because everything was dedicated to vaccine development. That situation has improved tremendously, but we are still seeing issues with plastic bags and product containers. Recently in the USA, we have seen a challenge in getting medical-grade CO_2 delivered to manufacturing facilities.

We have addressed those challenges by working with our primary, secondary, and tertiary sponsors to provide uninterrupted acquisition of raw materials. We have also assessed having a larger standing inventory. When we first resumed activities in our manufacturing facility, we were seeing lead times approaching 40–50 weeks for a customized product, even for simple things such as longer tubing or different connectors. We had to evaluate whether customized products were worth it, especially if there was an equivalent product we could get off the shelf.

It is also paramount to have good relationships with your suppliers. You need to be able to pick up the phone and talk to someone about your needs and have a supplier who recognizes those needs and strives to fulfill them. That is critical to being successful today.

JYS: Coming out of the pandemic, many manufacturers, including Corning, have learned how critical it is to have a secure supply chain. When a specific therapy or program advances to the next stage or shows promise, you may suddenly require a lot of material. If using a platform with a long lead time, that can become a challenge. My advice to anyone working in cell and gene therapy is to let your suppliers know ahead of time when you anticipate ramping up production so everyone can prepare and secure inventory. Do not be shy about informing your suppliers that there a potential for a surge so that we can assist in securing that inventory.

Q What advances in terms of platform, process, or partnerships have you been most excited about in recent years, and what's on your wish list for the future?

NT: Cancer is the leading cause of death worldwide. We have seen a lot of advances recently, particularly in the case of immunotherapy, with a few curative drugs on the market.

DOI: 10.18609/cgti.2023.054

We have checkpoint inhibitors like PD-1 and CTLA-4, and recent CAR T cell therapies approved by the FDA, which are all very effective. I am amazed by the progress in the cancer field.

On the gene therapy side, we have a couple of technologies, including CRISPR/Cas9, that have increased precision. Novel technologies are making a great impact on patients' lives.

In future, I would like to see more precise and selective gene editing tools. For cell therapies, I would like to see strategies to reduce the manufacturing cycle. Currently, the cell therapy manufacturing cycle ranges from 9 days to 4 months. A few companies are working on reducing manufacturing time, and a goal in the space is to bring this time down to 1–3 days.

In addition, we need to automate processes, either the unit operations or the whole process end-to-end. I would like to see end-to-end automation in manufacturing processes to increase the manufacturing success rate and manufacture more drugs in a shorter time. In this area, a few companies are working on making a GMP-in-a-box approach.

WS: Over the last couple of years in cell and gene therapy, we have seen movement into licensed products. This has been a seminal moment for the field. The success that we are seeing in the CAR T cell therapy arena has been amazing.

One thing that would be beneficial would be a movement away from autologous T cells towards off-the-shelf allogeneic products that accomplish the same goal. That would eliminate some individual product manufacturing. We would also like to see broader personalized medicine, where a patient's novel epitopes expressed on their cancer can be reverse-engineered to make a CAR T cell or a product. To achieve this, we need to make small-dose products in a robust and fast manner.

JYS: One recent advance I have seen is platforms becoming smarter. Corning is creating platforms with smart sensors that can provide a feedback loop and let researchers know if something is trending differently from expectations. Having these types of tools that you can add to platforms gives a greater sense of security that your process is advancing correctly and that you will be able to achieve your desired output in a more controlled manner. On my wish list is the ability to fully remove the scientist from the production room and allow remote control of what is happening.

AFFILIATIONS

William Swaney
President of Manufacturing, Expression Therapeutics

John (Yoshi) Shyu
Director, Global Scientific Applications, Corning Life Sciences

Nikhil Tyagi
Director of Cell Therapy Process Development, Center for Breakthrough Medicines

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The authors declare that they have no conflicts of interest.

Funding declaration: The authors received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: This article is based on an Expert Roundtable discussion, which was recorded on Feb 9 2023, which can be found here.

Expert Roundtable conducted on: Feb 9 2023; **Revised manuscript received:** Apr 18 2023; **Publication date:** Apr 21 2023.

DOI: 10.18609/cgti.2023.054

Multiply your cells, not your work.

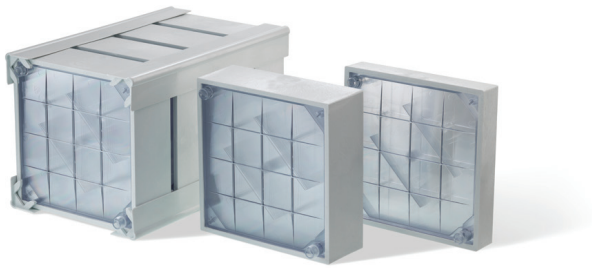
Roller Bottles

HYPERFlask® Vessels

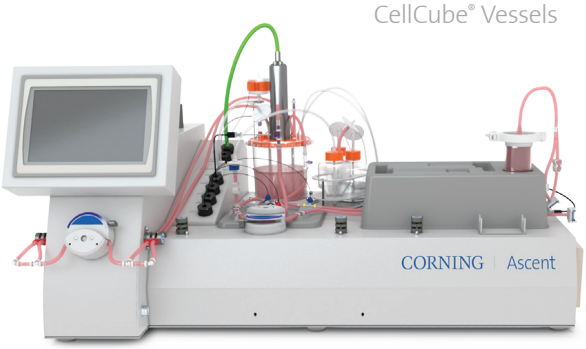
Microcarriers

CellSTACK® Chambers

HYPERStack® Vessels



CellCube® Vessels



Ascent® FBR System

Scale up with Corning Adherent Cell Culture.

You're ready to scale up your next big idea. To take your novel gene or cell therapy from the lab to production. But you can't spend time or money converting to a costly and complex suspension platform. You need to produce the highest yield in the most efficient way possible. Corning's proven portfolio of innovative, closed-system, adherent cell culture products work in concert to help you streamline your workflow. So you can scale up quickly, safely, and cost effectively.

To learn more, visit **www.corning.com/celltherapy.**
Scale up with confidence. And Corning.

CORNING

FASTFACTS

Viral vector production guide: part 3. Standardizing the manufacturing process

James Cody, Associate Director, Technical Sales, Charles River Laboratories

There are various strategies used in the manufacture of viral vectors, but each method has components that can be standardized, such as the cell line, plasmids, and production platform. To facilitate regulatory review and approval, it is necessary to consider the eventual commercial process and scale at the start of a program.

To streamline the adeno-associated viral vector (AAV) manufacturing process, implementing quality by design can maximize efficiency by identifying critical quality attributes (CQAs) and critical process parameters (CPPs) as early as possible. Relying on a platform approach can reduce potential manufacturing variability. Standardizing QC methods as early as possible is key as strong analytics are critical for monitoring process performance and confirming product quality.

STANDARDIZING AAV PRODUCTION AND TESTING

As shown in Figure 1, regardless of production strategy or serotype, there are various factors that can be standardized in AAV production and testing from input materials (plasmids, MVB, or stable cells), through both upstream and downstream purification to analytical testing.

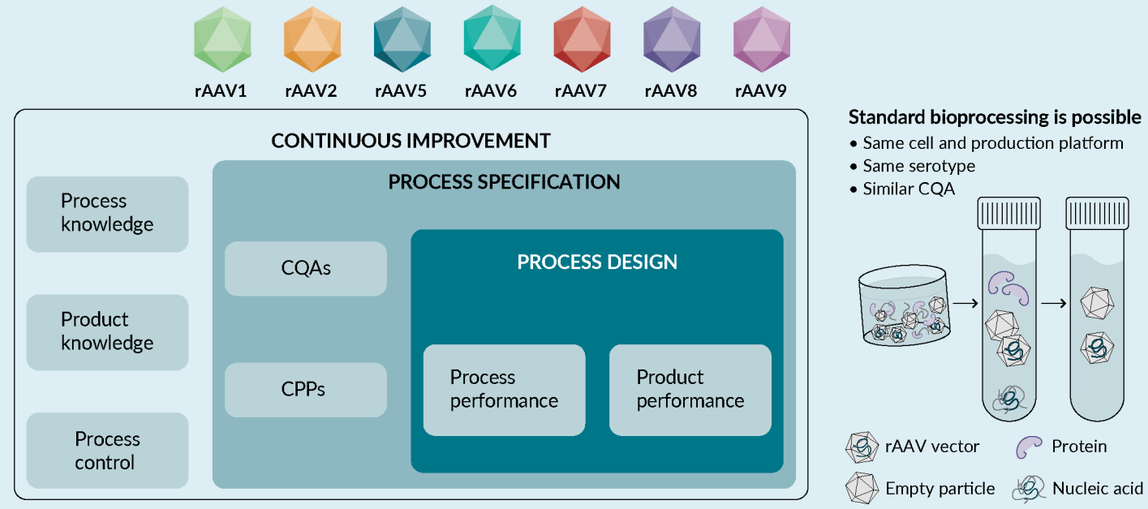
IMPLEMENTING QUALITY BY DESIGN (QBD)

Considering that there are several different AAV serotypes, process design should be based on both process and product performance **(Figure 2)**. These performances help determine the process specification, including the necessary CQAs and CPPs. These in turn are based upon the continuous improvement of process and product knowledge, as well as process control, as an established process is altered to improve quality and mitigate risk. Standard bioprocessing is possible in terms of having the same cell and production platform, the same serotype, or a similar CQA.

Figure 1. Factors for standardizing production and testing in the AAV workflow.

PLASMIDS AND HELPER VIRUS	UPSTREAM PRODUCTION	DOWNSTREAM PURIFICATION	ANALYTICAL TESTING
• Consistent large-scale production of plasmid DNA • MVB of helper virus available • Stable cell lines with RC and helper	• Bioreactor CPPS • Transfection agents • Transfection time • Clarification and harvest • In-process testing	• Affinity columns • TFF options • UF/DF • Formulation buffers • Final vialing choices	• In-line testing • Standardized qPCR/ddPCR test for serotypes • Standardized CQAs for in-process and final products

Figure 2. QbD and standardized bioprocessing.



PLATFORM APPROACH

Plasmid DNA is a critical raw material, which benefits from being produced using a standardized approach to reduce batch-to-batch variability. This could include a phase-appropriate approach or an off-the-shelf approach. Other considerations for standardizing production include using scalable upstream and downstream processing platforms.

STANDARDIZING QC METHODS

Most QC release tests rely on standardized or compendial test methods, though some product-specific methods must be developed. Establishing analytical methods early allows for the collection of more reliable data, which is critical for monitoring process performance and product quality. Phase-appropriate QC testing, including having qualified assays for Phases 1 and 2 and validated assays for later stage clinical and commercial, will save both time and cost. Using qualified assays during process development will give more consistent results and provide greater confidence in the data. Titer is especially important, as switching titer methods during development can change expected yields. If any assays are transferred, the transfer must be robust (same methods, reference material, equipment, acceptance criteria).

Cell & Gene Therapy Insights 2023; 9(2), 265; DOI: 10.18609/cgti.2023.040

FASTFACTS

Advancing AAV gene therapy development for the treatment of neurodegenerative diseases

Carina S Peritore, Product Manager, Neuroscience Discovery, Charles River Laboratories

Adeno-associated virus (AAV) is one of the safest vectors for targeted gene therapy due to its low immunogenicity and strong tissue tropism, with each AAV serotype able to transduce different cells or tissue types. This poster explores the benefits in using AAV for the treatment of neurodegenerative diseases.

AAV-DRIVEN GENE THERAPIES AS POTENTIAL TREATMENTS FOR CNS INDICATIONS

Gene therapies provide major advantages compared to other therapeutics for CNS which makes them ideal for neurodegenerative treatments. A key advantage is that proteins, which can be directly targeted by gene therapies.

AAV vectors are favored for *in vivo* studies due to their low immunogenicity and reduced oncogenic risk, However, the blood–brain barrier can make IV delivery of AAV to the brain highly inefficient and there is a risk of immune-mediated toxicity. Direct intracerebroventricular (ICV) injection is being attempted, but only allows localized delivery.

Figure 1. Comparison of AAV titer for AAV6 and AAV9 serotypes resulting from various delivery methods to mouse CNS.

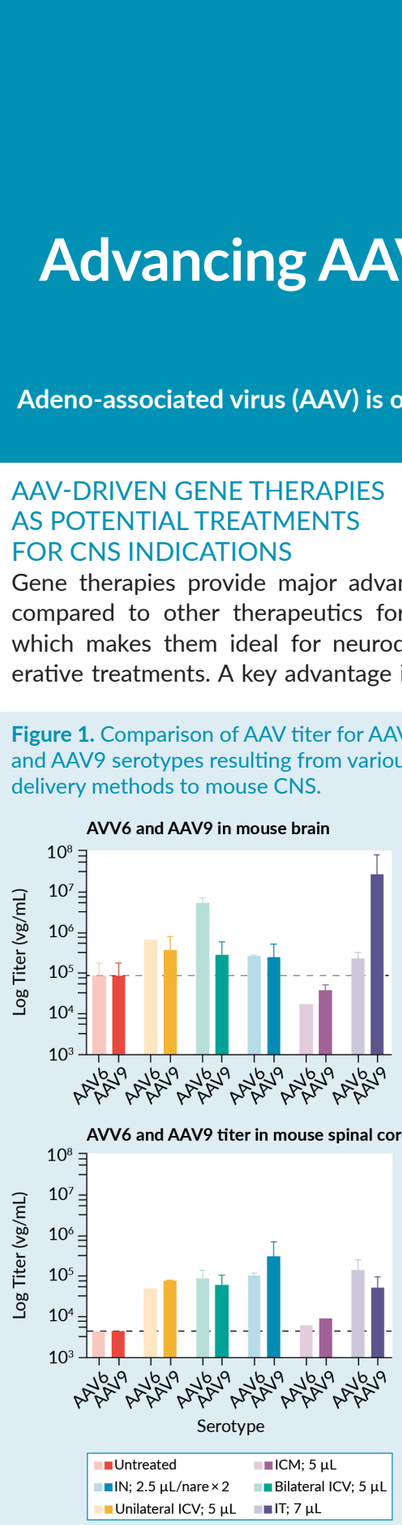
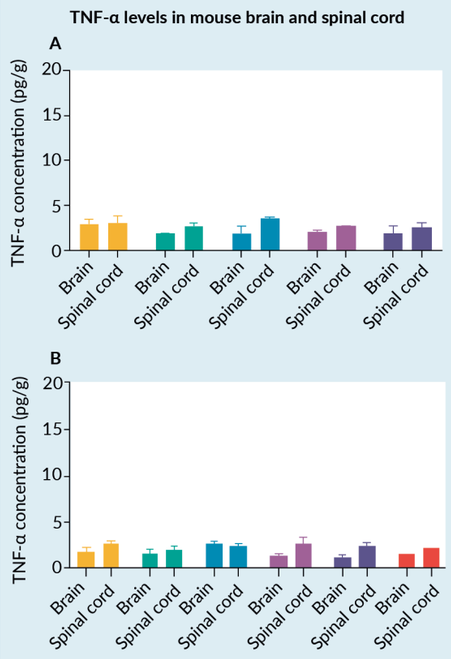


Figure 2. Immunogenicity to AAV6 (A) and AAV9 (B) serotypes based on route of delivery.



AAV vectors are delivered intracellularly. Although this has its challenges, successful delivery can modify protein production at the source and is advantageous with neurodegenerative disease as most of these pathologies occur intracellularly. Neurodegenerative diseases are caused by dysfunctional genes or small numbers of pathogenic

BIODISTRIBUTION OF AAV SEROTYPES IN MOUSE MODELS

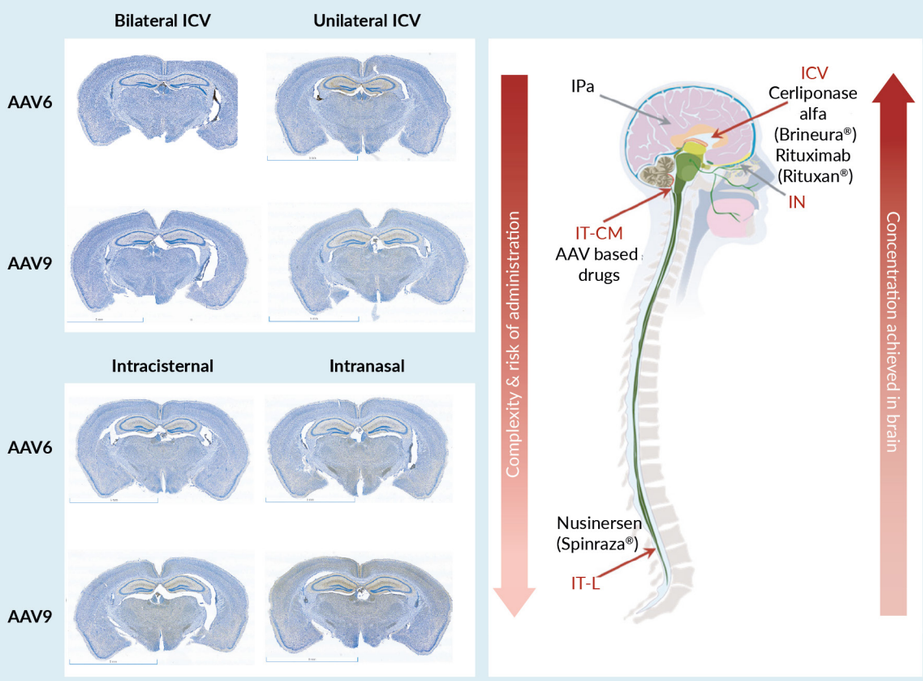
AAV9 is known to target CNS tissues with high neuronal tropism. This study investigated whether AAV6 was also suitable as a potential vehicle for gene delivery to the CNS and compared the two serotypes.

As shown in **Figure 1**, the highest titer for AAV6 serotype was achieved with bilateral ICV infusion while for AAV9, lumbar intrathecal (IT) injection was the most successful delivery method.

Proinflammatory cytokine levels were investigated and measured for acute and early inflammatory response to the viral load injected or infused into the mouse CNS. **Figure 2**. show that these levels did not increase for either serotype, or for any of the routes of administration tested.

With the use of staining methods and immunohistochemistry, it was found that brain transgene expression for AAV6 and AAV9 were similar for bilateral ICV, intracisternal (ICM), and intranasal (IN) **(Figure 3)**.

Figure 3. Comparison of transgene expression of AAV6 and AAV9 serotypes.



CONCLUSIONS

- The efficiency with which AAV vector is delivered to the CNS is dependent on both the serotype and delivery route used
- It is possible that both AAV6 and AAV9 are safe vectors for CNS gene delivery
- The brain expression for AAV6 and AAV9 were similar when utilizing bilateral ICV, ICM, and IN administration methods

CELL & GENE THERAPY INSIGHTS

Cell and Gene Therapy Insights 2023; 9(2), 267; DOI: 10.18609/cgti.2023.041

In partnership with: charles river

CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Challenges of developing anti-drug antibody & concentration (PK or PD) assays for liposomal transgene enzymes on an automated immunoassay platform

John Chappell & Issa Jyamubandi

Gene therapy can be used to permanently correct genetic disorders by delivering a transgene product into the nucleus of affected or alternative cells. One of the biggest challenges faced by the gene therapy bioanalytical sector is the lack of a true reference material for transgene products. As a result, in most assays, an alternative commercially available therapeutic (for example, an enzyme replacement therapy product) can be used as a surrogate for the transgene enzyme. This article discusses approaches taken in order to monitor the concentration of the expressed transgene product and any associated immunogenicity.

Cell & Gene Therapy Insights 2023; 9(3), 241–251

DOI: 10.18609/cgti.2023.036

Transgenes are expected to have very low levels of immunogenicity; therefore, immunogenicity assays against transgenes themselves are not widely developed. Nonetheless, it is important to monitor any potential case of immunogenicity. Current regulatory guidelines are not applicable to transgene products, which adds to the challenge. The following case study describes the considerations and challenges encountered when developing and validating an assay for transgene lysosomal enzyme products. Since enzymes are sensitive to changes in pH and salt, specific buffers were required to maintain their optimal

configuration to ensure all types of anti-drug antibodies (ADA), including neutralizing antibodies (Nabs), are detected.

The same level of challenges was also encountered during the development of an assay to determine the transgene product concentration, mainly due to the lack of a true reference standard and the presence of the equivalent endogenous molecule in healthy and disease matrices. Therefore, measurement of the transgene concentration could not be described as a true pharmacokinetics (PK) assay and does not conform to current the PK guideline/guidance, requiring an approach more aligned with a pharmacodynamics (PD) assay with a well-defined context of use.

THE GYROLAB® PLATFORM FOR IMMUNOASSAYS

Gyros Protein Technologies has supplied the market with proprietary high-performance nanoliter-scale immunoassay platforms, used by scientists in leading pharmaceutical, biotech, contract research organization, and contract manufacturing organization companies since the beginning of 2000 [1].

The core of Gyrolab technologies includes the range of compact discs (CD), which are the site of the immunoassay reaction, or the equivalent of a microtiter plate, involving precise volume definitions. Samples and reagents are transferred to the 15 nL affinity flowthrough column, which allows various assay formats. Detection involves laser-induced fluorescence to give an indication of assay binding. This provides good assay sensitivity in addition to a broad dynamic range.

The Gyrolab immunoassay platform is fully automated and has an integrated workflow to enable increased productivity and reproducibility. Volumes are at a nanoliter scale to save both reagent and sample. The platform enables short turnaround times, with a full assay taking approximately 1 h to perform. The platform's broad dynamic range reduces the number of dilution steps in the assay. Reduced matrix effects facilitate assay transfer in all stages of drug development.

The open platform results in a flexible approach supporting many different assay designs, including sandwich, PK, bridging, and indirect assay formats in one-, two-, three-, four- or five-step processes. Gyrolab methods supporting different assay designs are available for download.

AN OVERVIEW OF AAV GENE THERAPY BIOANALYSIS

Adeno-associated viruses (AAV) are replication-deficient, non-enveloped viruses. They hold promise for use in gene therapy due to their low immunogenicity, and their demonstrated long-term gene expression. AAV can transduce a wide variety of tissues, with over 11 well-known human viral serotypes. AAV are not known to cause any human diseases. Their mode of action is described in Figure 1.

In terms of bioanalysis, one key factor is to understand if there is anything that prevents AAV from entering cells i.e., pre-existing antibodies against AAV. Through assay development, it has been demonstrated that in some cases, over 70% of pre-existing antibodies are present for some types of AAV. It is important to pre-screen to avoid the presence of those candidates in a trial, or for the removal of those pre-existing antibodies.

Once the AAV is inside host cells, the genome material from the AAV needs to be monitored, usually by qPCR.

TRANSGENE QUANTIFICATION

The quantification of the transgene does not follow current PK guidelines. The lack of a true reference standard is the main challenge, meaning that the only quantification that can be done on a transgene product is relative. To quantify a product, an alternative available therapy that mimics expected transgene must be used as a calibrator.

Due to this, the quantification of transgene products is more aligned with biomarker

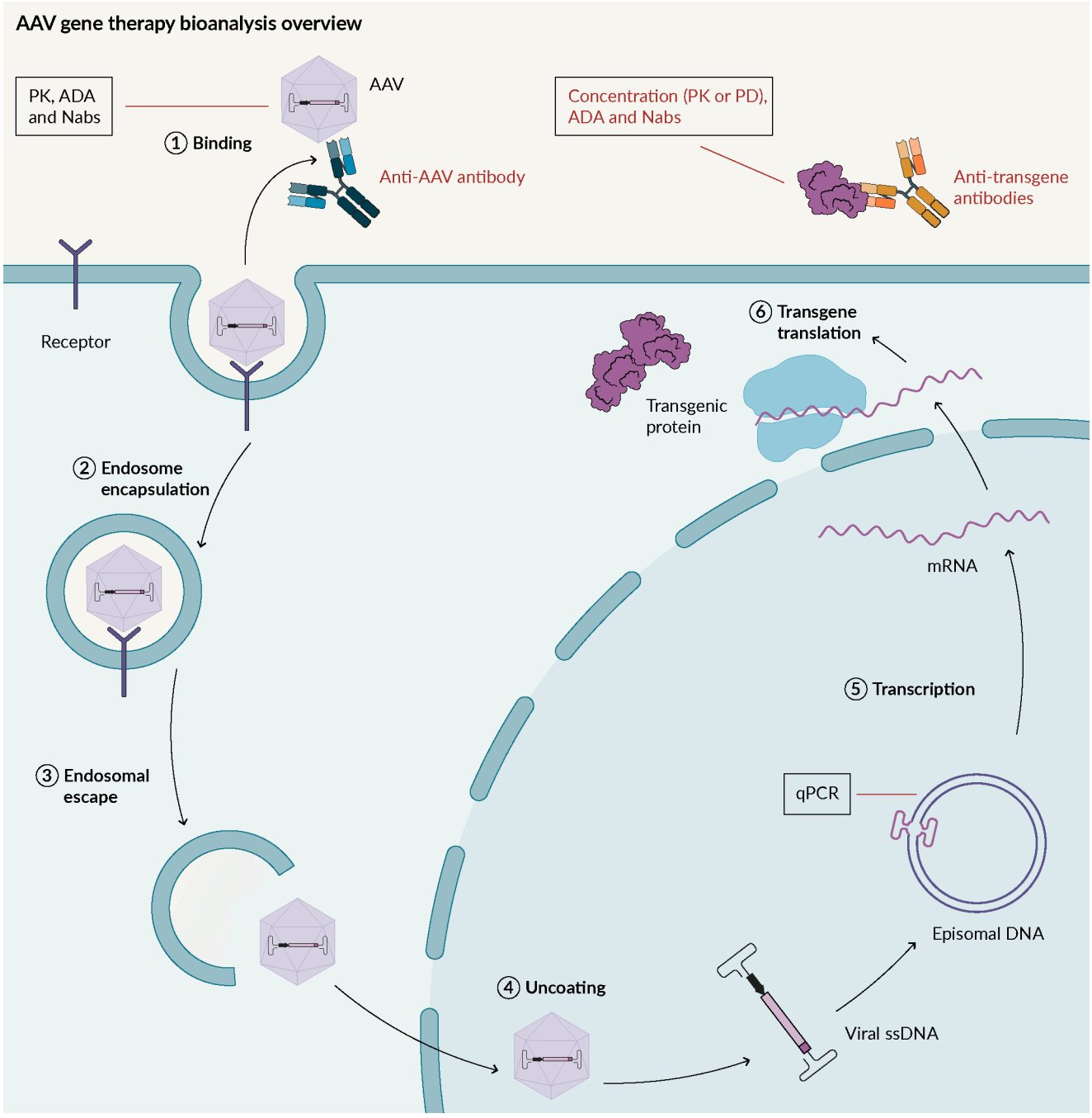
DOI: 10.18609/cgti.2023.036

assessment, or PD. As a result, the context of use is more important for transgene assessment. For example, there may be an interest in looking for the change from baseline to physiologically relevant levels.

An important factor to consider is that expressed transgene product will closely resemble the endogenous protein, and it can be difficult to distinguish them. Liquid chromatography–mass spectrometry (LC–MS) is

FIGURE 1

Adeno associated virus mode of action.



AAV; Adeno associated virus: ADA; Anti-drug antibodies; Nabs; Neutralizing antibodies; PD; Pharmacodynamic: PK; Pharmacokinetic.

advisable if the expressed protein has a unique structure different from that of the endogenous counterpart. However, the LC–MS method might lack some of the sensitivity that a ligand-binding assay might provide. In every case, parallelism and stability are crucial. Therefore, the assay is only fit for purpose and only becomes relevant once parallelism has been demonstrated in the sample containing transgene product.

Key considerations for transgene quantification include sensitivity, which can be as low as the nanogram or picogram range. However, transgene expression is expected to be consistent, so the required dynamic range of an assay is small. A stepwise format is the standard go-to for most quantitative methods. However, in cases of transgene products where the assay range is not large, a homogenous assay format can be accepted. In the examples described here, a homogenous assay format was demonstrated to provide the best sensitivity, and the best parallelism compared to a sequential format.

When working with transgene enzymes, it is important to optimize the buffer, as enzymes can be prone to conformational change. Conformational change can lead to epitope-masking, resulting in poor recovery (Figure 2).

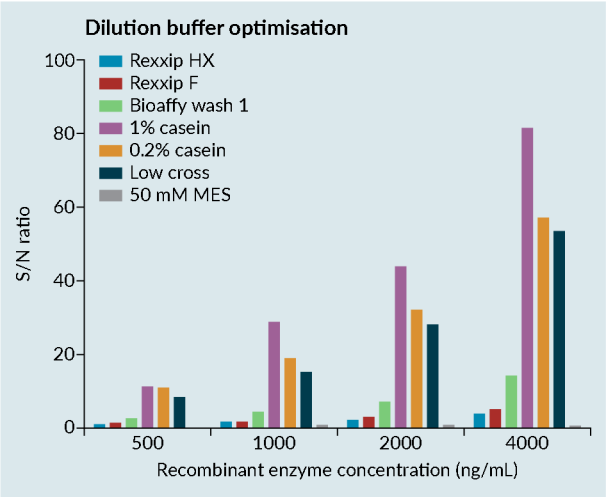
Figure 2 shows the dilution buffer optimization assessment of a transgene enzyme in various buffers, conducted by the contract research organization Drug Development Solutions.

Rexxip™ buffers, which are known to work well for antibodies, were demonstrated to not be compatible with enzymes, likely due to the buffer salt content. Simple buffers such as casein improved the sensitivity of the assay. However, casein has poor solubility and the precision in the assay was relatively poor.

When developing an assay, it is important to consider temperature, pH, and salt concentration, which are especially vital when using enzymes, as they can play a key factor in conformational change. The treated matrix was prepared by heating to 56°C to denature the endogenous enzyme. In this case, heat-treated matrix improved parallelism compared to the use of any other surrogate matrix.

FIGURE 2

Enzyme optimization consideration.



Platform comparisons were performed to evaluate the sensitivity and precision of Meso Scale Discovery (MSD) and the Gyrolab platform (Figure 3). It was demonstrated that the Gyrolab offered slightly improved sensitivity and precision compared with MSD. In addition, the Gyrolab gave much better parallelism data, compared to the MSD (Figure 4). This led to the work being taken forward on the Gyrolab. As discussed previously, parallelism of the endogenous enzyme was crucial to demonstrate that a ligand-binding assay method is fit for purpose.

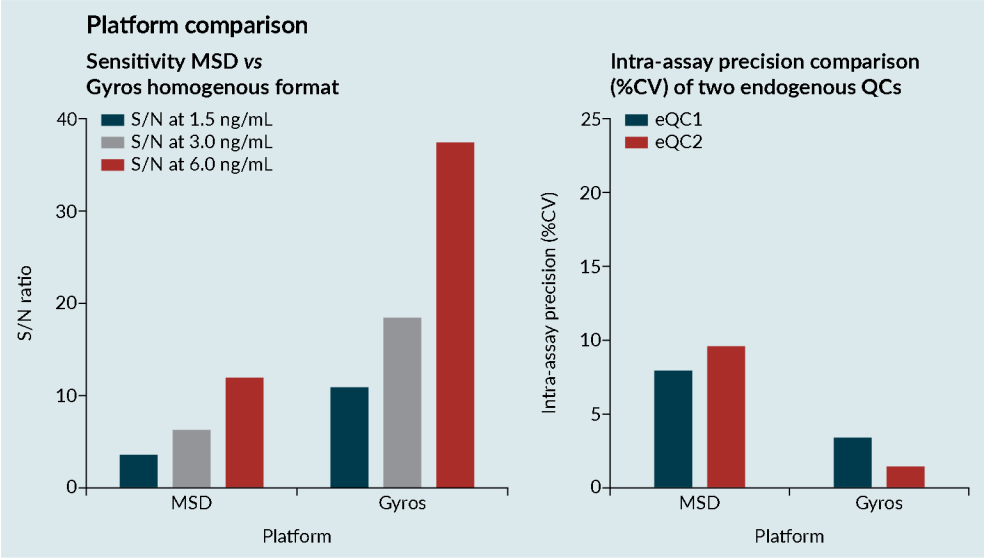
ADA METHOD ASSESSMENT

As with the PK assay, there is no current guidance on immunogenicity assessments of transgene products. The current FDA guideline clearly stipulates that it does not relate to cell and gene therapy products. While developing these methods, it was necessary to look elsewhere to establish the most appropriate means to assess the immunogenicity of transgene products.

DOI: 10.18609/cgti.2023.036

FIGURE 3

Platform comparisons: MSD versus Gyrolab platform sensitivity and precision.



CV; Cell viability: MSD; Mesoscale discovery; S/N; Signal-to-noise; QC: Quality control.

At Drug Development Solutions, the same approach has been adopted for assessing anti-transgene product antibodies as for a normal protein (Figure 5). In some cases, it is important to assess if conformational changes have affected the enzyme and that the catalytic unit is not affected. If there is any evidence that this is the case, then it is important that any sample analyzed in the ADA assay is also analyzed in a NAbs assay.

Prior to ADA assay development, it was known that the transgene endogenous circulating concentration was going to be present at a very low level – this provided the flexibility to be able to develop a homogenous or stepwise assay format. Both formats would have required the need to conjugate the enzyme. Enzyme conjugation provides additional challenges such as the need to be kept in the right medium, to avoid epitope masking.

Method development was initially carried out using the Gyrolab xPlore, and transferred to the Gyrolab xP or xPand as they allow the use of more CDs. When the conditions were selected, the capture was dissolved in Rexxip F, the detection was diluted in Rexxip F, and the solution was diluted five-fold in Rexxip ADA. When the method was transferred, an assessment of multiple CDs demonstrated loss of the assay signal.

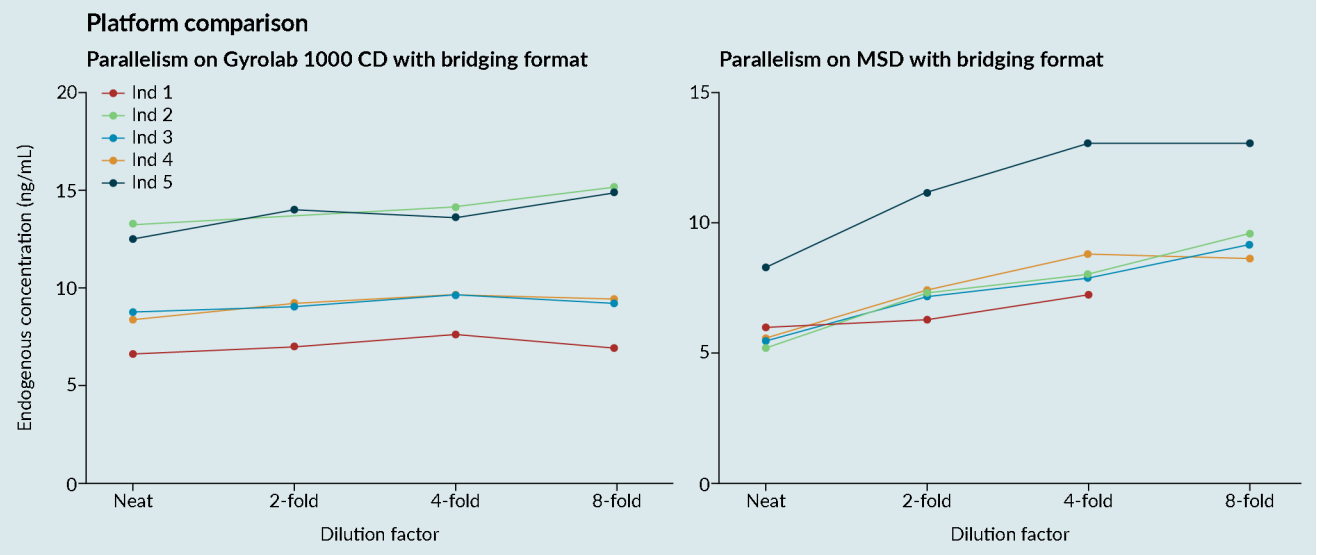
To investigate what caused these issues, Drug Development Solutions approached Gyrolab to assist in determining which buffer was appropriate. The first point of call was looking at a buffer with an isoelectric point closer to the enzyme of interest. With the Rexxip Hx, a similar signal deterioration over multiple CDs was found, with high coefficients of variation (CVs) more than 20% also observed. It was apparent throughout these experiments that the loss of signal was also associated with higher precision failure.

Given that the buffers were the root cause of this problem, the simplest buffer from Gyrolab, the wash station solution 01, was then assessed. This demonstrated a stable signal; however, the baseline was high, leading to poor sensitivity. The increase in signal also improved the CVs. These data clearly demonstrated that the Rexxip buffers were causing issues with the enzyme, which helped in terms of optimizing other buffers.

In this case, casein was found to improve assay sensitivity and reduce the baseline to the desired levels. However, precision was an issue for casein. The Gyrolab team advised

FIGURE 4

Platform comparison: MSD versus Gyrolab platform parallelism.



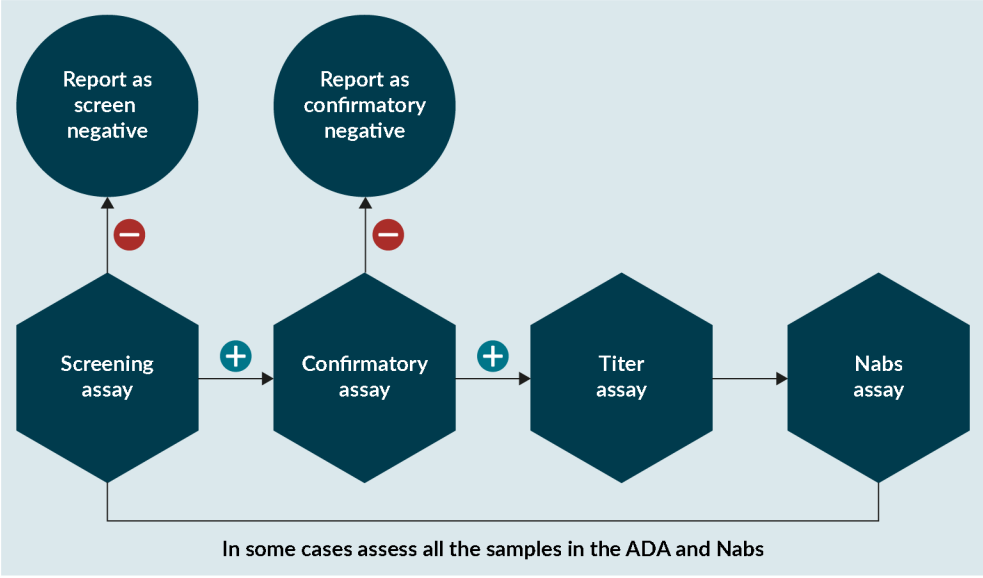
increased centrifugation and an additional mixing step to improve the precision issue, which was successful.

CONCLUSIONS & RECOMMENDATIONS

It is crucial to consider that enzymes are especially sensitive to temperature, salt, and pH change. Care is required to avoid missing a positive result as a result of enzyme conformational change. Where possible, assess conjugated enzymes using a potency/NAbs assay to confirm activity. Before transferring from the Gyrolab xPlore to the Gyrolab xP, extended sample stability and extended reagent stability should be assessed. All CDs should be spun simultaneously to reduce challenges with reagent and sample stability and shorten the run time.

FIGURE 5

An approach to assess anti-transgene product antibodies.



DOI: 10.18609/cgti.2023.036

REFERENCE

1. Gyros Protein Technologies. Installations worldwide.

ASK THE AUTHORS


Issa Jyamubandi, Principal Scientist, Alliance Pharma and John Chappell, Director of Scientific Support, EMEA and Asia Pacific,Gyros Protein Technologies (pictured left to right) answer your questions about anti-drug antibody and concentration assays for liposomal transgene enzymes

Q Was acid dissociation used in the ADA assay, and if not, how was the required drug tolerance achieved?

IJ: For most of the ADA assays, we tend to add an acid dissociation, mainly to improve drug tolerance. In this particular case, when we tried the use of drug tolerance, we observed that the signal baseline was significantly higher. The reason for this was that the assay was affecting the enzyme, causing aggregation and an increased baseline.

We noticed the heat was de-naturing the enzyme, as described in the PK assay. Therefore, we used heat to make the assay more transgene tolerant, which worked in this case.

Q What is the preferred approach to the transgene product PK assay?

IJ: The use of that surrogate heated matrix worked for us. The advantage of the heated matrix is that all the endogenous matrix is removed, and it keeps the matrix similar to the matrix that is deficient in the desired transgene product.

This leads to improved assay sensitivity, and the other matrix interference can be resolved at the same time. You do not want to get into sample analysis and realize you have poor parallelism. You must remember the transgene product is not what we are using as a calibrator. We want to ensure that the matrix interference you see in development is similar to that you see in the sample analysis.

Q You mentioned that the method for transgene quantification assessment can only be classified as fit for purpose after sample analysis. Can you elaborate on that?

IJ: This comes back to the use of reference material. For the PK assay, we have to use a well-characterized reference material and have the right paperwork.

In this case, we are using products that mimic expected transgene products. The quantification and validation results are not what we expected for the transgene product. We can claim the assay is fit for purpose once we get the samples that contain the actual transgene, quantify them, and carry out the parallelism to ensure the condition of these samples mirrors that of the surrogate product that we have used for validation.

Q When developing the transgene quantification assay you saw slightly higher sensitivity, better intra-assay precision, and parallelism with the Gyrolab platform compared to the MSD platform. Have you experienced these differences in results between the technologies for other types of assays?

IJ: With the PK assays that we develop internally, we adopt the principle of always trying to develop these assays on multiple platforms, so we can make better decisions.

In this case, the Gyrolab was better than the MSD. One of the biggest advantages of the Gyrolab platform is you can develop your assay quickly compared to the use of the MSD.

In some cases, MSD can give you the required sensitivity, but in this case, even though the MSD may have provided better sensitivity, the method had poor parallelism. The Gyrolab provided acceptable data in terms of parallelism, which was key in terms of what we were looking for here.

Q Why was the homogenous assay format beneficial for PK analysis when compared to the stepwise assay format?

IJ: For any form of PK, we usually prefer to use a stepwise format. The stepwise format is key in terms of avoiding things like the hook effect. With PK, you can have a very wide dynamic range. You do not want to underestimate the concentration of your drug in the sample.

In this case, we knew that the dynamic range required was narrow based on the context of use. Homogenous assays are specific, and in some cases also more sensitive. In this case, it was proven to be the most sensitive method, and the parallelism on the homogenous format was much better. As a result, we chose the homogenous format.

Q Are there any available guidelines on which type of CD to use depending on the assay?

JC: It depends on what the assay sensitivity requirements are. Most of the time, we recommend that assay development starts on either the 200 or the 1000 nanoliter CD.

DOI: 10.18609/cgti.2023.036

This ensures that you can screen reagents and test the assay range. If you need a very sensitive assay we would recommend you use the 4000 nanoliter CD.

If you want to measure very high concentrations, you may want to look at a 200-nanolitre CD. The advantage of the 200-nanolitre CD is it has 112 additional structures. This allows you to screen more reagents.

Q For the ADA assay, what was conjugated?

IJ: The same problem is present for either a PK assay or an ADA assay – you must find an alternative material that mimics your transgene product, and then assume that your transgene product will behave similarly. My best advice would be to get the exact transgene product that you want, by getting the transgene elsewhere. That would be the perfect case scenario for PK.

Q What percent of casein worked for the ADA?

IJ: We used 1% casein. It is worth bearing in mind that casein at 1% is still not very soluble. 1% gave us the desired baseline, but we had to implement additional strategies such as increased centrifugation and additional mixing step to be able to achieve the desired precision in the assay.

BIOGRAPHIES

JOHN CHAPPELL has approximately 25 years of experience in the Contract Research industry supporting both preclinical and clinical drug development. He has specialized in supporting biological compounds from an analytical perspective e.g. Pharmacokinetic, Immunogenicity and Biomarker analysis. He is particularly interested in validation requirements and ensuring that data generated will be acceptable to the regulatory authorities. He has spoken at many international conferences on various topics including Oligonucleotide analysis, Biomarker Analysis, Immunogenicity and the analytical support of Biosimilar programs. He now leads the Application Support and Service teams for Gyros Protein Technologies where he is responsible for customer service and technical support in Europe and the Asia Pacific regions. John has been a user of the Gyrolab® system for over 10 years so will use this experience to help customers. He is a Fellow of the Royal Society of Chemistry and was involved in the American Association of Pharmaceutical Scientists (AAPS) Biosimilar Committee that has prepared papers on Pharmacokinetic and anti-drug antibody assays.

ISSA JYAMUBANDI received a BSc in Biomedical Sciences from the University of Coventry and a PhD from the University of East Anglia (UEA) developing targeted peptide drug conjugate therapies (PDCs) for melanoma. Issa has over 10 years' experience developing and validating anti-drug antibodies (ADA), neutralizing antibodies (Nabs) and PK assay using various platform including the MSD, Gyrolab and various absorbance, fluorescence and luminescence platform. Issa is subject matter expert in the development and validation of ADA and Nabs assays for challenging therapeutic products ranging from small peptides (<5kDa), bispecific, ADCs, enzymes, Pegylated therapies, AAV gene therapy and transgene products.

AFFILIATIONS

John Chappell
Director of Scientific Support,
EMEA and Asia Pacific,
Gyros Protein Technologies

Issa Jyamubandi
Principal Scientist,
Alliance Pharma

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The authors have no conflicts of interests.

Funding declaration: The authors received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: This article is a transcript of a webinar, which can be found here.

Webinar recorded: Jan 26 2023; **Revised manuscript received:** Mar 7 2023; **Publication date:** Mar 30 2023.

DOI: 10.18609/cgti.2023.036

Accelerating time to insight

Automate your immunoassays with Gyrolab® and compress cell and gene therapy development timelines

Learn how to automate immunoassays like ELISA

GYROS PROTEIN Technologies