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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT

Next steps in the gene editing revolution

GUEST EDITOR

Sven Kili, Saisei Ventures

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NEXT STEPS IN THE
GENE EDITING REVOLUTION

SPOTLIGHT

A guided tour of the gene editing landscape

EDITORIAL

"As we work to build an editing toolbox, choosing the right technology for the right indication at the right time in the right patient is paramount."

Guest Editor of our 'Next steps in the gene editing revolution' edition, **Sven Kili** (Partner, Saisei Ventures), takes us on a guided tour of the gene editing landscape, highlighting recent milestones, approvals, emerging technologies, and ongoing challenges in the space. As breakthroughs in precision, personalization, and platform diversity begin to reshape what is possible for patients and developers alike, we reflect on where the field stands today and what it will take to realize the full potential of gene editing.

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A NEW ERA OF PERSONALIZATION

The recent case of the first patient treated with a personalized CRISPR-based gene editing therapy at the Children's Hospital of Philadelphia (CHOP) marks a historic milestone for the field [1]. This exciting development demonstrates, perhaps for the first time, that truly individualized gene therapy is not only possible, but achievable within a clinically relevant timeframe.

Until now, what we have often referred to as 'personalized' gene therapy has typically involved tailoring a standard correction to a patient's own cells–personalized

in source, but not in design. This case marks a significant step beyond that, with personalization at the level of the genetic defect itself, and that makes it genuinely groundbreaking. The speed at which this was accomplished reflects the maturity of our genomic understanding. For a growing number of conditions, we now know the nature of the precise genetic defect, how to correct it, and how to translate that correction into a therapeutic modality on a one-to-one basis.

The speed of this achievement also challenges the status quo of regulatory organizations. We saw during the COVID-19 pandemic how quickly the MHRA, for example, was able to review and approve the AstraZeneca vaccine. There was widespread discussion about how to make that kind of regulatory agility routine to the benefit of patients. While we have yet to fully realize that ambition, this case shows that, under the right conditions, it can be done.

It is important to remember that this personalized approach is not suitable for every patient, nor even for every individual with an ultra-rare disease, but for a significant number of ultra-rare conditions, this level of personalization may well be feasible. This is not a replacement for existing therapies, rather, it is an additional tool in our toolbox for treating genetic diseases and improving the lives of patients.

CRISPR/Cas9 TECHNOLOGY AT A TURNING POINT

The recent approvals of an *ex vivo* CRISPR/Cas9 editing therapy have been a long time coming [2,3,4]. While the scientific progress in this space has been extraordinary, clinical translation has lagged behind, hindered by a range of technical and regulatory challenges. Credit is due to the organizations and companies that have successfully navigated these hurdles to bring CRISPR-based therapies into the clinic and, ultimately, to approval.

To achieve broader clinical and commercial success, the technology must now evolve beyond its current applications. We need to see CRISPR/Cas9 and its variants used routinely across a wider range of indications. That means improving the platforms to reduce off-target effects and editing efficiency, both of which remain a significant challenge. It also means expanding our understanding of where and how these tools can be applied. So far, most clinical applications have focused on single edits, which are relatively straightforward. Much like the early *ex vivo* gene therapies that targeted well-characterized diseases such as ADA-SCID, metachromatic leukodystrophy, and Wiskott-Aldrich syndrome, the first *in vivo* CRISPR therapies have rightly gone after the low-hanging fruit.

The next step is to reach higher, for more complex diseases that may require more than one genetic modification. This is where lessons from allogeneic cell therapies become relevant. In immuno-oncology, for example, a single edit is often insufficient. Multiplexing (making multiple edits simultaneously) becomes essential to the successful generation of a hypo-immune cell.

However, the current CRISPR/Cas9 system presents a technical barrier: the editing machinery is large and delivering it into cells consumes significant vector space. As a result, we are typically limited to one edit per viral vector. Attempting multiple edits often requires multiple vectors, which complicates delivery and limits efficiency. To overcome this, we need to develop more compact editing systems that support multiplexing. This is where newer gene editing technologies may offer a solution. With smaller molecular footprints and greater flexibility, these next-generation tools could enable the kind of complex editing required for more ambitious therapeutic targets.

Off-target effects and unexpected immune reactions remain key safety

concerns in CRISPR-mediated gene editing. We don't yet fully understand all the implications–some may have long-term implications we are not recognizing, while others might be clinically irrelevant but still cause alarm. To address this, we need better analytics, tools that help us understand not just the changes we are making, but their biochemical and clinical relevance, now and in the future. We also need a multitude of efficient, targeted, and accurate guides, which will work for different indications.

As we develop these newer technologies, both CRISPR-related and other technologies, it is critical to use the correct editing modality for the correct indication. As we work to build an editing toolbox, choosing the right technology for the right indication at the right time in the right patient is paramount.

EXPANDING THE TOOLBOX: NOVEL PLATFORMS AND TECHNOLOGIES

The new editing technologies we are seeing (prime editing, base editing, gene writing, epigenetic editing, etc.) are all extremely exciting. Importantly, these are not replacements for CRISPR-Cas9; they are additions. They allow us to fine-tune the way we make edits or changes within a cell, whether those changes are permanent or temporary, expressed or not, *ex vivo* or *in vivo*. This gives us many more choices in the toolbox. Once we understand the strengths and weaknesses of each technology, supported by better analytics, we will be able to develop safer, more effective, and potentially more permanent therapies for patients, by applying the correct tool at the right time.

The use of AI and machine learning will be critical, given the large volumes of data being generated in developing and analyzing the various gene editing technologies and applications. Additionally, the data generated by next-generation analytics, single-cell RNA sequencing and other newer analytical systems, is also extensive. We will struggle to analyze all of it in a way that produces meaningful insights with the human brain alone. AI and machine learning will help us understand the results, find connections that we would otherwise miss, and ultimately support better outcomes for patients.

ASSESSING THE INVESTMENT AND FINANCING LANDSCAPE

From an investor perspective, gene editing is no longer special in and of itself. Thousands of researchers around the world are using these technologies every day. From a scientific perspective, editing is still remarkable. But to investors, the days of financing 'cool' science projects are over. Most of the investors who remain in the cell and gene therapy space are well educated in the science; they understand the development challenges and are reluctant to fund projects that do not clearly translate into therapies that will make a difference in patient's lives. What they want to see is how editing can be applied to create safe, effective therapies that solve real problems. Companies must show how their technology enables a therapy that is safer, faster, cheaper, or more durable than existing options.

It is critical for companies to understand where their technology fits within the broader therapeutic landscape. Not just within cell and gene therapy, but in comparison to small molecules, biologics, surgical, and non-surgical approaches. Investors are not looking for something that is simply more advanced; they are looking for something that is better. That is what companies need to focus on: the application of the technology in a way that makes a meaningful difference to patients' lives.

Currently, there is no single financing model that has clearly emerged as the best path forward. We are still recovering from the hyper-investment period during

COVID-19, when large amounts of capital flowed into the sector. This money was often from generalist investors who did not fully understand the challenges of developing cell and gene therapies, let alone drug products. As those investors withdrew after the pandemic, it left a gap that we are still working to fill. It is worth noting that the level of investment during COVID-19 was an aberration and should not be seen as any form of normal. Early-stage venture investment is returning to pre-COVID-19 levels. For companies developing new editing technologies, the hurdles are significant. They must first demonstrate safety, then show that their platform is better than existing technologies and finally prove that it can work in humans and deliver real clinical benefit.

To support this, companies should focus on developing better assays and understanding of the strengths and weaknesses of their technology. No single platform will be superior in every context, but each will have its place. It is the role of the company to find that place and convince investors of the value of that place and approach.

Partnerships are also critical. Collaborating with pharmaceutical companies can help offset development costs. Working with assay developers or analytics partners can strengthen the overall package. It is about building a foundation and showing not just that the technology works, but how it works, why it works, and where it works best.

As the financing environment begins to ease, and we are starting to see early signs of that, these targeted efforts will help attract more investment. For now, companies must focus on generating the most important, decision-driving data and building the right collaborations to support their progress.

BUILDING REGULATORY AND CMC READINESS

From both a regulatory and a CMC perspective, gene editing remains a relatively new technology, especially with the emergence of epigenetic editing, prime editing, base editing, and gene writing. There is always a degree of lag in figuring out how to manufacture these therapies efficiently, how to scale them, and how to develop the right assays and analytics to support quality and consistency.

This is where partnerships become important. Companies developing new technologies should seek collaborations that bring in analytical expertise. These partnerships can support CMC process development and help ensure that manufacturing is as efficient and robust as possible. We are starting to see some service providers offering "selectively pre-edited" cell lines for sale as a way to shorten development timelines and provide a more cost-effective option to cell engineering.

On the regulatory side, the same principle applies. Regulators are not embedded in the day-to-day development process. They do not see the blood, sweat, and tears that go into building these therapies, which is why early engagement is critical. Developers must help regulators understand the technology: its strengths, its limitations, and its potential.

The regulators want to learn. Agencies such as the EMA, MHRA, FDA, and PMDA have all expressed a desire to make these therapies available to patients. In order to do that, they need to understand them. Bringing regulators along on the journey, educating them, and giving them the opportunity to ask questions will be essential to enabling timely, pragmatic, efficient, and patient-focused regulation.

PREPARING FOR COMMERCIALIZATION: START EARLY, THINK BROADLY

The transition to commercialization is critical. For many years, companies viewed regulatory approval as the finish line. That mindset is thankfully beginning to shift.

Approval is not the end; it is just a milestone. Commercialization requires a completely different approach, and companies must begin preparing for it much earlier than they think.

Start early. Then, start planning even earlier than that. Even during preclinical development, when defining the target product profile, companies should be thinking about the commercial environment and ecosystem in which the therapy will be used. Understand how the therapy will be used, not just in clinical trials, but in real-world settings. It is essential to consider the broader treatment landscape, as your therapy will not exist in a vacuum. It will be used alongside or in place of small molecules, biologics, surgical procedures, and other treatment options. Understand where it fits and what makes it better than the current standards of care. This insight can then be used to refine your commercial strategy.

Market knowledge is also key. The US is not the same as the UK, or Germany, or Japan. Each market has its own dynamics, and companies must understand those differences early. Engage with payers as soon as possible. Like regulators, they need time to understand the therapy, especially when it comes with a high price tag.

Finally, build relationships with key opinion leaders. They can provide critical insights into how the therapy will be used in practice, how it fits into treatment protocols, and how to position it effectively. Their input can shape development and support successful adoption.

Gene editing is an incredibly powerful set of tools. The onus is on us as the CGT ecosystem to demonstrate this by developing them in a way that benefits patients and moves human medical care forwards in a very crowded competitive field.

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BIOGRAPHY

Sven Kili is a respected KOL in healthcare and biotechnology, boasting over 20 years of experience. He began as a surgeon in the NHS, where he gained valuable insights into patient care. Transitioning to cell and gene herapies (CGT), he played key roles in leading the development, approval and commercialization of both a cell therapy and the first *ex vivo* gene therapy. With expertise across all stages of CGT development, he has built companies and teams in both large pharma/ biotech and startups.

Sven is a partner at Saisei Ventures, a boutique therapeutic venture investment firm focussed on creating and growing innovative companies, including with a focus on the Japanese CGT ecosystem. He also heads his consulting firm, Sven Kili Consulting Ltd, offering strategic guidance to organizations in the biotech and healthcare sectors. Most recently he served as the Chief Development Officer for CCRM (Centre for Commercialization of Regenerative Medicine) and OmniaBio.

Sven actively contributes to industry boards and committees, including the BIA CGTAC, Innovation Hubs for Gene Therapy, ISCT, and ARM. He holds a visiting chair at University College London, where he heads the steering committee for a post-grad degree in Cell & Gene Therapy Manufacturing and Commercialisation. His dedication to advancing regenerative medicine and CGT is reflected in his multifaceted roles and ongoing efforts to shape the future of healthcare through innovation and strategic collaboration.

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CELL & GENE THERAPY INSIGHTS

NEXT STEPS IN THE
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COMMENTARY

History, opportunities and challenges for protecting gene therapies: perspectives from private practice and industry

Jennifer O'Farrell and Damien O'Farrell

Recent years have seen an explosion of advanced therapies, many of which are based on personalised immune cells, individually edited using gene editing techniques. This technological advancement presents great opportunities to patients and the healthcare industry as a whole, but also presents challenges to established commercial principles, including logistics, pricing, and protection. The patent system provides companies with period of exclusivity in return for the disclosure of their invention. This well-established system allows companies to recoup some of the R&D costs associated with innovation, offsetting some of the costs incurred by innovation which does not reach the market, for example due to low success rate of developing drugs to the clinic. Here we explore the opportunities and challenges facing companies as they look to develop robust patent protection around a gene therapy, considering the challenges and opportunities facing companies looking to protect innovation in this space, and how this differs from protecting traditional therapies, e.g., therapeutic antibodies.

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PATENT PROTECTION FOR TRADITIONAL THERAPIES

For traditional therapies, composition of matter patents (those claiming a product, e.g., an antibody, a nucleic acid or a small molecule) are generally accepted to be the most valuable. Such patents provide protection for any use of an active agent, and even patents only narrowly covering an active agent can be extremely valuable if they cover a commercial product.

Follow on patents covering formulations, methods of treatment, production methods and combination therapies can provide additional protection and reward for innovation during a product's development, providing additional patent term to

ensure that a commercial product is protected beyond the 20-year term of an initial composition of matter patent.

Supplementary Protection Certifications (SPCs) in Europe, patent term extensions in the US, and similar provisions in other jurisdictions, can also provide additional patent term for a commercial product. This additional protection is designed to compensate companies for delays in achieving regulatory approval, and SPCs can extend the term of a patent by up to five years (with an additional six months available if paediatric trials are conducted). Such protection is generally narrower in scope than the granted patent, and is limited to the approved product. Nevertheless, such additional protection can represent an extremely valuable form of protection as they come at the end of the lifetime of a patent, at which time a product is usually at its commercial peak.

The protection available for traditional therapies is well understood. This allows companies to have a degree of certainty about the protection they will be able to obtain for their innovation, the licensing strategies they may adopt and the third-party patents they may need to consider.

PATENT PROTECTION FOR GENE THERAPIES

The patent strategies suitable for protecting gene therapies can differ from those used to protect traditional therapies.

Gene therapy is, of course, a broad term covering a wide range of treatments, including gene editing technologies, *ex vivo* autologous immune therapies, *ex vivo* gene therapies for genetic disorders and tumor-infiltrating lymphocyte therapies (TIL therapy).

For the purposes of highlighting the challenges and opportunities of protecting innovation in this space we will focus here mainly on aspects which are common to all of these therapies. As explained below, numerous aspects of a gene therapy may be protected, and a robust IP strategy should consider each of these.

The patient's cell

TIL and *ex vivo* autologous therapies, by their nature, rely on a patient's own cell as the therapeutic product. For ethical reasons it is long established that naturally occurring cells cannot be protected, and where the therapeutic product is essentially an unmodified cell, e.g., TIL therapy, it may therefore be challenging to obtain patent protection for the cell per se.

Patent protection for modified cells is possible and patents have been granted for modified cells which are intended to be administered therapeutically. However, such modifications must meet the patentability criteria and therefore must be both novel and inventive. In the case of gene therapies for genetic disorders, this can prove problematic, as discussed below.

Gene of interest

As with modified cells, obtaining patent protection for therapeutic gene sequences is possible, but again such gene sequences must meet the patentability criteria, that is they must be both novel and inventive.

The therapeutic gene sequence used for *ex vivo* immune therapies is usually a novel CAR or T cell receptor (TCR). In these cases, patent protection may be available for the nucleic acid sequence itself, the sequence of the protein it encodes, a vector comprising the nucleic acid sequence, and a modified cell comprising the novel sequence. Provided such sequences have not been disclosed before, obtaining such protection should not be overly problematic.

Obtaining patent protection is, however, more difficult when it comes to gene sequences used in gene therapies for genetic disorders. In this case, the therapy requires a patient's cell to be modified to correct a faulty gene or introduce a functional copy

of a gene that is mutated or missing in the patient. The corrected sequence is usually the wild-type sequence of the gene of interest, and is therefore unlikely to be novel and thus unpatentable. In addition, in many jurisdictions, naturally occurring gene sequences per se cannot be patented because they are considered to form part of the human body, for which patenting is restricted on ethical grounds.

However, novel modifications to such gene sequences may render them patentable, and patent protection should be considered in such circumstances.

Vectors

Many of the gene therapies discussed herein rely on viral vectors to deliver genetic material to a patient's cell. Commonly used viral vectors include lentiviral vectors and adeno-associated viral vectors. Both of these vectors were originally patented around the turn of the millennia, and so most of the underlying patents have now expired.

It is possible to obtain protection for new and inventive viral vectors, but as there is now a large body of literature describing modified viral vectors, such protection is likely to be fairly narrow and may be limited to, for example, a specific coat protein modification, or a particular addition/modification to the viral backbone sequence. Nonetheless, such protection is worth pursuing if a new viral vector exhibits desirable characteristics over known vectors, e.g., specific tropism, serum half-life, etc.

Non-viral vectors are also being used increasingly due to their reduced immunogenicity and cytotoxicity. Non-viral vectors utilise naked DNA, and require innovative solutions to introduce the DNA into cells successfully.

Manufacturing process

It will be appreciated that gene therapies are inherently more complicated than traditional therapies. As a minimum, such therapies require extraction of a patient's cells from their body (relying on specific techniques, e.g., apheresis, tumour resection, bone marrow extraction, etc.), treatment and handling of the cell's outside of the body (the complexity of which is dependent on the type of gene therapy) and then re-administration of the cells to the patient.

Each step in the overall manufacturing process represents a potential opportunity to capture patentable innovation, and care should be taken to ensure that all innovation is appropriately protected as a gene therapy evolves. Whilst the gene sequence and specific vector are unlikely to change from pre-clinical work through to Phase 3, it is possible that the manufacturing process will develop, leading to the potential for obtaining additional protection. Issues arising during scale-up of manufacturing, modifications to achieve GMP, or as a result of feedback from regulatory bodies, can require innovation, which in turn represent potential patentable inventions for anything from the bioreactors to transport containers to media formulations.

As with traditional therapies, follow on patents covering formulations, methods of treatment, modifications of production methods, and combination therapies can add value to a product's patent portfolio.

FREEDOM TO OPERATE AND LICENSING

As described above, gene therapies are inherently more complicated than traditional therapies. With that complexity comes a significant opportunity for innovation, from the product per se (gene sequence, vector, etc.) through to the complex manufacturing process (cell extraction, cell processing, formulation, logistics, delivery to patient, etc.). However, this increased opportunity for obtaining protection comes with increase difficulty of insuring freedom to operate across the same scope.

For a small molecule or antibody therapy, once freedom to operate of the therapeutic product *per se* is established, a routine manufacturing process may be used to produce the final product. However, for a gene therapy one or more of the multiple steps involved in producing a gene therapy product may be covered by a third-party patent. It is important in these cases to ensure that a robust freedom to operate search is conducted as early as possible for both the product per se and the complete manufacturing process. Such a review will aid in increasing awareness of issues which may occur prior to commercialisation and may also allow product development to pivot to take into account significant freedom to operate roadblocks.

Of course, any discussion of freedom to operate naturally leads into a discussion of licensing as a mechanism for resolving such issues. In an ideal world, parties will, having generated their own patents, be in a position to cross-license as a way of resolving a freedom to operate issue. However, even in the absence of cross-licensable patents, it may be beneficial to start any third-party licensing discussions as early as possible to provide business certainty.

A NOTE ON KNOW-HOW

Not all innovation which arises during the development of a gene therapy product will reach the level of innovation required for patentability. However, there is real value in the kind of specialist know-how which goes into the design and manufacture of a gene therapy product. Careful consideration should be given to how such know-how is managed and captured. Particular care should be taken to ensure that such know-how is not lost through unintentional disclosure, or poor IP protection policies. A thorough IP policy should consider the management of know-how, in addition to the protection of innovation through more formal routes, e.g., patents.

COMPETITOR MARKET ENTRY

When considering the strength of a patent portfolio for a gene therapy product, it will be beneficial to take a more nuanced approach than is the norm for a small molecule or antibody therapy.

Once protection, including both patent protection and data exclusivity, for a traditional therapeutic has expired there will be a number of generic and/or biosimilar companies capable of producing and marketing the product, and this often occurs as soon as protection expires.

However, at this point in time, the equivalent manufacturing capacity and expertise is not available in the gene therapy space. This is particularly true in the *ex vivo* immune therapy space, where there are few, if any, manufacturers, with manufacturing capacity sat idle waiting for the opportunity for a gene therapy product patent to expire. This means that in the gene therapy space the expiration of the final patent covering a product may not represent the date on which competition arises and this may, in fact, occur considerably later. Parallels can be drawn with the vaccines space, where the patent cliff is not nearly as steep as that observed with traditional therapeutics such as small molecules and antibodies.

Gene editing

Although we have included gene editing in the general discussion of gene therapies above, there are a number of additional considerations that are worth calling out specifically for this technology, and these are discussed further below.

Gene editing methods: platform protection

Cell editing methods may constitute a platform having a variety of uses, including the development of a number of gene therapy products. Patent protection for such

platforms may be widely applicable and can therefore be extremely valuable.

The worldwide patent battle between the Broad Institute and University of California Berkley surrounding the CRISPR technology has been well documented. Here the key issue was which institute had first managed to modify eukaryotic cells using CRISPR, and the chronology of the data arising from the various institutes was analysed extensively as the dispute progressed in multiple jurisdictions. Ultimately the European Patent Office considered that University of California Berkley had not adequately described the CRISPR method in their patent application before the publication in *Science* in 2012, leading to revocation of the patents.

For innovation in a crowded field, there is a balance to be struck between filing a patent application at an early stage to ensure you are ahead of your competitors, and delaying long enough to ensure you have sufficient data to support the patent application. This applies particularly to platform inventions, where obtaining an early filing date may be key to commercial success.

The key to protecting a platform is crafting a patent application which is broad enough to adequately cover the platform, but which is also sufficiently supported by the data available. There is often a tendency in a developing field to draft a patent application broadly in an attempt to cover future developments of the platform, but this can result in a weaker application and may ultimately lead to a loss of protection.

A more cautious approach would be to narrowly, but robustly, protect the platform in its initial form and plan to file further applications as the platform develops. This approach can increase the patent protection covering a platform, which may be attractive to investors and potential licensees. However, it requires constant analysis of developments being made to the platform, and the data available to support these developments, illustrating the importance of getting your patent strategy right from the outset and being vigilant as the technology develops.

Gene editing methods: product protection

Composition of matter patents have traditionally been considered the most valuable and most straightforward to enforce, but deciding on the product to protect and obtaining valid protection for products in the gene therapy field may not be straightforward. It may be prudent to try and protect multiple different products, such as the gene sequence, the modified cell and the constructs.

Obtaining patent protection for a gene sequence itself is attractive since this goes to the core of a gene therapy.

However, obtaining protection around a gene sequence may not be straightforward since the innovation really resides in identifying a disease-causing mutation within a wild-type gene sequence, and protecting the disease causing mutation may be of limited use.

Protecting the corrected gene sequence will also be challenging if the wild-type gene sequence is known, since such patent claims would lack novelty even if ethical considerations would allow these to be protected.

The patent protection available for naturally occurring cells is also limited in view of ethical considerations. Of course, modified cells are, by definition, not naturally occurring. However, protecting modified cells may be challenging if these cells are indistinguishable from naturally occurring cells, the generation of which is, of course, the aim of such therapy. Further challenges may arise in relation to defining such cells, unless a range of distinct biomarkers can be identified.

An attractive option may be to protect the constructs required to modify a cell.

Such constructs would not be naturally occurring, and therefore should not face an ethical barrier to protection. From a reimbursement perspective such products may be attractive as these are likely to form the commercial product underpinning a therapy. However, since such constructs are not actually a medicinal product in their own right, obtaining SPC protection is likely to be very difficult.

In order to have the best possible chance of obtaining a composition of matter patent it would be advisable to consider filing patent applications encompassing a number of different products involved in the therapy, some of which are discussed above.

Gene editing methods: protecting the method

Method claims are another useful route to obtaining protection for an individual therapy. Such method claims would be distinct from method claims directed to a platform since they are likely to relate only to a single therapy, or group of related therapies.

Method claims may provide valuable protection and would not suffer from some of the restrictions discussed above in relation to composition of matter patents.

One important consideration for a method claim is who will infringe the claim. Ultimately a claim needs to be infringed by a single party in order for a patent to be easily enforceable, and for a multiple-step method, with steps potentially occurring across a number of geographical sites, this may not be straightforward.

Another consideration relates to the restrictions to patenting methods of medical treatment, particularly in Europe, which are intended to ensure that medical professionals can perform their roles without risking patent infringement. Medical use claims in the form "X for use in treating disease Y" represent a simple reformulation of method of treatment claims, but these claims require a therapeutic compound. Whilst this is straightforward to define for a traditional therapy, it is much more difficult for a gene therapy where producing the product from a patient's own cells forms the substantial part of the therapy.

Provided they are well thought through, and avoid the pitfalls mentioned above, method claims may provide useful protection for gene therapies, and should be considered in foundational patent applications.

CONSIDERATIONS FOR THE FUTURE

As the gene therapy field matures and more therapies are approved, the companies involved will look to recoup some of their investment in R&D.

Obtaining robust patent protection around the assets will form an important part of the commercialisation of gene therapies, and there are considerations for doing so which extend beyond those which apply to traditional therapies. Whilst this requires investment in a patent strategy, it also provides opportunities for companies shrewd enough to obtain valid protection.

An increase in the number of approved therapies will lead to greater requirements for third party licences as well as a consideration of a company's own freedom to operate position. In a world where valid, robust patent protection is likely to look slightly different from that obtained for traditional therapies, an understanding of the principles behind obtaining and retaining appropriate protection will be increasingly important.

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CELL & GENE THERAPY INSIGHTS

NEXT STEPS IN THE
GENE EDITING REVOLUTION

SPOTLIGHT

From CRISPR to DNA writing: expanding the gene editing toolbox

INTERVIEW

"It is amazing to witness the speed by which discovery can now be translated into practice."

Abi Pinchbeck (Editor, BioInsights) speaks to **Laura Sepp-Lorenzino** (Scientific Advisor and former CSO, Intellia Therapeutics) about the evolution and future of gene editing, focusing on CRISPR-based therapies. They also discuss major scientific advancements, clinical successes, delivery challenges, and the promise of emerging technologies, such as base editing, prime editing, and DNA writing.

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Q What are you working on right now?

LSL I have been doing research in biotech and pharma for over 30 years: from basic biology to translational science to drug development.

I feel privileged to have worked across all therapeutic modalities. I started with small molecules, and after a short stint in vaccines and biologics, I moved into the RNA interference space. After that, I worked with other platforms including mRNA, gene therapy, and gene editing.

As I look to the next phase of my career, I am increasingly focused on corporate strategy and long-term value creation, particularly through board service and strategic advisory

"Within 10 years of the discovery of CRISPR...Intellia took that biochemical breakthrough and turned it into a new class of medicines for *in vivo* genome editing."

roles. I am also committed to being a vocal advocate for the biopharmaceutical sector. Having served on the board of the Alliance for Regenerative Medicine and recently elected to the board of the American Society of Gene & Cell Therapy (ASGCT), I see a vital opportunity and responsibility for our field to educate the public and policymakers about the transformative potential of biopharma to improve patient lives.

Q With decades of experience in the biopharmaceutical industry, you have witnessed the evolution of gene editing technologies firsthand. How has the field evolved in terms of scientific/tech advancements, and where do you think we are headed in the next decade?

LSL When I first started, it was all about small molecules and biologics. Then we began thinking about the potential of genomic medicines, and how to overcome significant technological hurdles. Through the contributions of many in academia and industry, real solutions were developed and put into practice—technologies that showed that we could, in fact, target the genome with a diverse toolbox of editors.

It is amazing to witness the speed by which discovery can now be translated into practice. Many of the solutions that were originally identified for one specific use have proven applicable across a wide range of other molecular entities. You can now combine these solutions much faster, enabling new technologies to move into preclinical proof of concept and then into the clinic.

Within 10 years of the discovery of CRISPR by Jennifer Doudna and Emmanuelle Charpentier, Intellia took that biochemical breakthrough and turned it into a new class of medicines for *in vivo* genome editing. That speed of discovery is incredible to me, and it paves the way for what is next. Innovation does not stop; now, we can build on this remarkable technology and platform and continue pushing forward to explore new avenues for treating disease.

Q How do you assess the current landscape for CRISPR-based therapies? What do you consider the most exciting developments in this space?

LSL At Intellia, we currently have two investigational *in vivo* CRISPR-based gene editing therapies being evaluated in three Phase 3 pivotal trials: nexiguran ziclumeran (or nex-z or NTLA-2001) for transthyretin (TTR) amyloidosis and lonvoguran ziclumeran (or lonvo-z or NTLA-2002) for hereditary angioedema (HAE).

We have seen that our lipid nanoparticle (LNP)-CRISPR *in vivo* platform works very well in humans, with predictable pharmacokinetics, pharmacodynamics, and tolerability. Importantly, they demonstrate deep, durable, and consistent knockdown across patients which based on emerging long-term data, is translating into clinical benefit. Nex-z and lonvo-z can be transformational for patients.

Recently, at the Peripheral Nerve Society meeting, the Intellia team presented 2 year duration data following a single dose of nex-z in patients with transthyretin amyloidosis with polyneuropathy. Although several TTR silencers have already been approved for that indication, they slow disease progression, but they do not stabilize or improve the disease. Additionally, these chronic disease therapies require lifelong administration, which comes with enormous cost. By contrast, nex-z treatment resulted in rapid, profound, and durable TTR knockdown, leading to clinically meaningful improvements in disease outcomes, including in patients who were progressing on a silencer (Patisiran). Similar positive results were obtained for patients with TTR cardiomyopathy, with compelling early evidence.

Similarly, longer-term follow up data of up to 3 years for lonvo-z in HAE showed that sustained knockdown, resulted in a 98% reduction in disease attack rate following a single intravenous dose with all patients, being attack-free for a median of 23 months, with no use of additional prophylaxis. These are exciting results and Lonvo-z is poised to become the first approved *in vivo* CRISPR therapy.

Beyond Intellia studies, I am also excited about what others in the field are doing. For example, Beam Therapeutics has shown promising results in alpha-1 antitrypsin deficiency. Additionally, Verve Therapeutics and CRISPR Therapeutics are both progressing in cardiovascular disease treatment. We are also starting to see the emergence of epigenetic editors. Hopefully, we will see positive data on chronic hepatitis B treatment. Furthermore, Arbor Biotechnologies is in the clinic for primary hyperoxaluria. It is encouraging that many of these companies are following the Intellia playbook: using LNPs *in vivo* to target diseases caused by a gene in the liver and demonstrating that they work. These are real solutions that can help real patients, and that is what matters.

Q What are some of the key hurdles that still need to be overcome to fully realize the promise of CRISPR in the clinic?

LSL The biggest hurdle to realizing the full potential of these therapies is delivery beyond the liver. If we want to address the full range of genetic diseases, the liver is just a small fraction. As a field, we need to figure out how to develop safe, efficacious therapies for extrahepatic targets. I have been encouraged by some of the early data we have at Intellia, and by what others are working on, but these programs are early research programs.

Furthermore, these therapies offer a potentially curative, single administration approach, distinguishing them from chronic treatments. As such they present compelling pharmacoeconomic advantages, particularly when administered early in the disease.

However, the field has yet to address the challenges surrounding commercialization, including reimbursement strategies and long-term payment models. The economic rationale is clear: for chronic conditions such as HAE, the lifetime cost of chronic therapies can exceed $500,000. In this context, the cost-effectiveness of a one-time, durable treatment becomes self-evident.

"I believe that *ex vivo* HSC editing will eventually be superseded by *in vivo* approaches targeting HSCs directly in the bone marrow. "

Q How will the field address remaining safety challenges in CRISPR-mediated gene editing, including unexpected immune reactions and off-target effects?

LSL Starting with precision and accuracy of the edit, it is crucial to select guide RNAs (gRNAs) for which you fully derisk the potential for off-target activity. Ultimately, it comes down to a benefit-risk assessment, and it will vary for each indication. The goal should always be to identify gRNAs that do not have confirmed off-target effects, even at multiples of the intended therapeutic dose.

Regarding immunogenicity, at Intellia, we use LNPs, which means Cas9 expression is very transient, minimizing the risk of triggering an immune response.

Another consideration is the immunogenicity of the edit itself. For example, if you are re-expressing a gene for which patients have no immunological tolerance, could that be a problem? Again, this will be disease-specific and must be validated before moving into the clinic.

Q Given your experience in both *in vivo* and *ex vivo* cell and gene therapies, could you share your perspective on the relative strengths and challenges of these two approaches?

LSL Together, *in vivo* and *ex vivo* approaches expand the reach of CRISPR-based therapies, but there are significant differences between them. *Ex vivo* and *in vivo* therapies cover fundamentally different indications.

For *ex vivo*, the work so far has primarily focused on accessible immune cells and hematopoietic stem cells (HSCs). CASGEVY, from CRISPR Therapeutics and Vertex Pharmaceuticals, was the first major example—an impressive effort in *ex vivo* editing of HSCs for sickle cell disease and beta-thalassemia. That said, the uptake of CASGEVY has not been strong. Some of these challenges include the complexity of process, the cost, and the patient journey. The need for preconditioning and the associated short-term and long-term impact, as well as the risks inherent to bone marrow transplantation, limit adoption. I believe that *ex vivo* HSC editing will eventually be superseded by *in vivo* approaches targeting HSCs directly in the bone marrow. Several companies are working on this now, and I am confident we will see progress.

When it comes to cell therapies in immuno-oncology or immune diseases, autologous therapies do work, but they come with multiple issues around scalability, cost, complexity, and time. This has been particularly challenging with CRISPR-based approaches. Allogeneic therapies taken to the clinic so far have not hit the mark because they have not addressed all the immune challenges necessary to truly make them allogeneic. Intellia has recently presented on what we believe would be a significant step forward in allogeneic therapies; however, the proof will be in the clinic, whenever that milestone is achieved.

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One of the responses to the limitations of CAR-T cell therapies has been a shift toward *in vivo* CARs, using LNPs and mRNA. I think the data we are seeing there, especially for autoimmune conditions, where the goal is to reset the B cells, has a high probability of both technical and clinical success.

Q What role do you think emerging gene editing technologies, like base editing and prime editing, will play in expanding the therapeutic applications of gene editing?

LSL CRISPR is an excellent genomic 'geo-locator' allowing developers to precisely identify a specific target sequence and then introduce the desired genetic change.

I am particularly excited about DNA writing because it offers enormous versatility. It goes beyond what a base editor can do because you can make all transitions, transversions, and perform small insertions and deletions, all with the same platform.

Beyond DNA writing, I am also excited about epigenetic editors. For example, in chronic hepatitis B, the ability to silence the virus and its integrated DNA, which is scattered throughout the genome, is very powerful.

Next is the ability to insert longer pieces of DNA, which is still in its infancy. The efficiencies are currently low, and there are more challenges to overcome. For instance, there are hurdles related to the use of DNA as a template, particularly around nuclear delivery and innate immune activation. However, I expect these problems to be tractable and solvable.

Q What are your hopes for the future of the gene editing field? And what would your advice be to the next generation of advanced therapy developers working on gene editing-based therapeutics?

LSL We are not merely developing new tools—we are creating medicines. This demands a thoughtful selection of indications and an unwavering focus on the patient.

Second, resilience is essential. In drug discovery, failure is common. But each setback is an opportunity to learn, adapt, and move forward with greater insight.

Third, collaboration is key. Breakthroughs happen at the intersection of disciplines. It is through teamwork and the integration of diverse perspectives that we catalyze transformative advances.

BIOGRAPHY

Laura Sepp-Lorenzino is a biotech executive with expertise in drug development, particularly in nucleic acid therapies. She currently serves on the board of directors of Taysha Gene Therapies, AskBio Biopharmaceutical, Sail Medicines, the Alliance for Regenerative Medicine, and the American Association of Cell and Gene Therapy.

Previously, Laura was Chief Scientific Officer at Intellia Therapeutics (2019–2025), where she played a pivotal role in shaping the company's strategic direction, fostering a robust and diversified CRISPR platform, and driving groundbreaking advances in in vivo genome editing. Under her leadership, Intellia demonstrated the first successful in vivo genome editing in humans and advanced two candidates into pivotal trials. Before Intellia, she served as VP, Head of Nucleic Acid Therapies at Vertex Pharmaceuticals, as well as VP and entrepreneur-in-residence at Alnylam Pharmaceuticals, and Executive Director of RNA Therapeutics at Merck & Co.

Laura Sepp-Lorenzino PhD, Scientific Advisor, former CSO of Intellia Therapeutics, Inc., Boston, MA, USA

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CELL & GENE THERAPY INSIGHTS

NEXT STEPS IN THE
GENE EDITING REVOLUTION

SPOTLIGHT

Rewriting the code: examining the promise and responsibility of gene editing

INTERVIEW

"Scientific progress means little if it does not translate into availability and equity in care."

On May 13, 2025, Abi Pinchbeck, Commissioning Editor, *Cell & Gene Therapy Insights*, spoke with **Bambi Jo Grilley**, Director, Clinical Research and Early Product Development, Center for Cell and Gene Therapy, Baylor College of Medicine, about the evolving landscape of gene editing. This conversation highlights scientific breakthroughs, regulatory challenges, and the urgent need to reduce costs and expand global access to ensure equitable delivery of transformative therapies.

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Q What are you currently working on?

BJG I work in regulatory affairs, supporting the advancement of research by ensuring compliance with regulatory requirements. At present, I am engaged in reviewing and preparing two IND applications for submission. Both involve first-in-human studies and represent exciting, innovative developments in the field.

I am fortunate to be based at Baylor College of Medicine, a highly active academic medical center, where I have the privilege of collaborating with outstanding scientists across a

"For many years, CGT has been predominantly cancer-focused. However, there is increasing interest in extending these technologies to autoimmune diseases..."

range of disciplines. My current focus is to ensure that these IND submissions meet all necessary regulatory standards. I anticipate that both applications with be submitted within the next month.

These INDs are particularly noteworthy as both target solid tumors using CAR-T cell therapy, one in adult patients only and one in both adult and pediatric patients. While CAR-T therapies have demonstrated substantial success in hematologic malignancies, the treatment of solid tumors has proven considerably more challenging. Each of these INDs represents a distinct approach to address the challenge of solid tumors. Additionally, there has been a dearth in approval of CGT products for pediatric patients so the inclusion of children in CGT research is incredibly important. Contributing to these efforts is a key priority within the broader cell and gene therapy (CGT) community.

In addition, although not directly related to my core responsibilities, I have observed a growing trend within the field towards applications beyond oncology. For many years, CGT has been predominantly cancer-focused. However, there is increasing interest in extending these technologies to autoimmune diseases, driven by our evolving ability to modulate the immune system with a reasonable degree of efficacy. This was a major topic of discussion at the recent International Society for Cell & Gene Therapy (ISCT) conference and represents a promising direction for future research and development.

Q Gene editing is increasingly becoming a primary focus within the CGT space. How has the field evolved, and what developments are currently shaping the space?

BJG Gene editing has indeed emerged as a central focus within the CGT landscape, and the field continues to evolve rapidly. Among the most transformative advancements is the application of CRISPR technology, which has fundamentally changed the approach to genetic modification.

There are two areas in which gene editing is currently demonstrating significant impact. The first is *in vivo* gene editing, and this approach is showing considerable promise in the treatment of single-gene disorders. Researchers such as Fyodor Urnov have advocated for the use of CRISPR to correct monogenic diseases, highlighting its potential to address the root causes of certain rare genetic conditions. However, the regulatory pathways are associated with developing therapies for rare diseases, where limited patient populations can complicate clinical trial design and commercialization.

The second major application is the *ex vivo* modification of immune cells, such as T cells used in CAR-T therapies. Currently, many of these modifications rely on viral vectors, which are costly and come with specific safety concerns. CRISPR-based gene editing presents a compelling alternative, offering the possibility of creating these cellular therapies without the need for viral vectors. This shift could lead to improvements in the safety profile of such therapies and a meaningful reduction in production costs, thereby improving accessibility and scalability.

"These challenges are even more pronounced in regions where genetic disorders such as sickle cell disease and beta-thalassemia are more prevalent...In many of these regions, there is limited or no access to therapies, not only due to cost but also due to the absence of regulatory infrastructure."

Q What are the current challenges in the gene editing space, particularly in patient access and cost? Additionally, where is the field making progress despite these challenges?

BJG The field of gene editing is advancing rapidly, yet several significant challenges remain. Among the most pressing is patient access, which is multifaceted, encompassing financial, regulatory, and scientific dimensions. One clear example is the recent approval of gene-editing-based treatments for sickle cell disease in the US. Despite their availability, adoption has been limited.

This is largely due to infrastructure limitations, as not all medical centers are equipped with the necessary technology to administer such therapies. In addition, the mechanisms for reimbursement remain unclear and, in many cases, impractical. The high cost of these therapies presents a substantial barrier. Even within the US, where the healthcare systems are relatively well-resourced, the likelihood of widespread insurance coverage is currently low, whether through private payers or government programs.

These challenges are even more pronounced in regions where genetic disorders such as sickle cell disease and beta-thalassemia are more prevalent, including parts of Africa and the Middle East. In many of these regions, there is limited or no access to therapies, not only due to cost but also due to the absence of regulatory infrastructure. Without formal regulatory frameworks, it is not feasible to distribute or administer these products safely and ethically.

Another critical barrier to access involves the toxicity profile of certain therapies. Many gene-edited products, particularly CAR-T cell therapies, carry significant risks that require administration within specialized healthcare facilities. These treatments often necessitate hospitalization, access to intensive care units, and availability of agents to manage adverse events. The need for such resources limits treatment availability to major medical centers, effectively excluding patients in rural or resource-limited settings.

Manufacturing also remains a key constraint. At present, most CGTs must be produced in GMP laboratories, typically located within academic medical centers. This requirement presents a logistical challenge for scaling up access, especially in underserved or remote areas. Expanding access will require innovation in decentralized or point-of-care manufacturing models that can operate outside elite research hospitals.

Despite these obstacles, the field is making measurable progress. There is a clear transition underway—from theoretical, lab-based work to real-world, approved clinical applications. The next critical phase involves scaling these innovations efficiently, safely, and equitably. It will require advancements not only in the science itself, but also in distribution, infrastructure, policy, and manufacturing solutions. Addressing these areas will be essential to ensure that the benefits of gene editing reach all patients who may benefit from them, regardless of geography or socioeconomic status.

Q While discussing the challenges in the gene editing space, global regulatory divergence is increasingly being raised as a concern. Do you have any thoughts on how we can navigate these regulatory complexities at an organizational level?

BJG Global regulatory divergence is indeed an important and increasingly visible challenge in the advancement of gene editing and CGT more broadly. As CRO at ISCT, I frequently engage in conversations about the need for global harmonization of regulatory standards. It is a complex issue, primarily because regulatory bodies such as the US FDA and the Japanese PMDA operate within the legal frameworks of their respective governments. These agencies do not create laws independently; rather, they implement and enforce laws enacted by their legislative bodies. As a result, achieving full harmonization would require coordinated policy changes across multiple national governments, which is inherently difficult and unprecedented in most fields.

That said, there is potential for progress, particularly in our field, given its unique and high-profile nature. There may be opportunities for global philanthropic or public health organizations, such as the Gates Foundation or the WHO, to spearhead coordinated international initiatives. If such organizations were to prioritize global access to gene therapies and engage directly with regulatory authorities, it could open the door to more streamlined approval pathways. For instance, if a product receives US FDA approval, other regulatory bodies might consider using that data to support their assessments, thereby reducing duplication of effort and expediting access.

At ISCT, we have made deliberate efforts to facilitate international regulatory dialogue. For the past three years, our annual Global Regulatory Summit has brought together representatives from regulatory agencies around the world to share perspectives, best practices, and experiences. This year, the summit focused on accelerated approvals, a topic that revealed a surprising degree of alignment across jurisdictions. Most agencies define the types of products eligible for accelerated pathways in similar ways. However, there remain significant differences in execution, particularly in review timelines. For example, in the US, accelerated approval might mean a six-month review period, while in other jurisdictions, timelines can range anywhere from 200 days to more than a year.

Despite these procedural differences, the underlying regulatory principles are increasingly aligned. The remaining challenge lies in mutual recognition. While agencies may evaluate products using similar criteria, they do not yet accept each other's conclusions. For example, an accelerated approval granted by the FDA is not automatically recognized by the EMA or UK MHRA which means that secondary reviews are still required. Greater trust and interoperability between agencies would be a meaningful step forward.

Looking ahead, the global distribution of patients with rare monogenic diseases may become a catalyst for regulatory collaboration. In cases where only a handful of patients exist worldwide, it becomes impractical for any single country to lead the regulatory process independently. Addressing such global patient populations may necessitate a more integrated and cooperative regulatory framework.

"There may be opportunities for global philanthropic or public health organizations…to spearhead coordinated international initiatives."

Q There has been growing discussion in HHGE, which has caused some concern within the CGT community. Could you outline the potential risks, any benefits, and explain why this topic is coming to the forefront now?

BJG The discussion around heritable human genome editing (HHGE) is closely tied to the advances we have seen in gene editing for rare diseases and monogenic disorders. HHGE represents the next phase of this scientific progression. The concept involves editing the genome at the embryonic stage, such that the correction is not only made in one individual but is passed down to future generations. Essentially, this moves us from somatic gene editing, of modifying the genetic makeup of an individual, to germline editing, which affects the individual's descendants as well.

The potential appeal is clear, as in theory, correcting a genetic defect at this early stage could eliminate certain heritable diseases across an entire family line. However, the concerns raised by the scientific and ethical communities are substantial and multifaceted.

From a scientific perspective, we cannot currently edit embryonic genomes with a high degree of certainty and safety. We do not yet fully understand the long-term implications of modifying specific genes. A gene alteration intended to prevent one disease could inadvertently increase susceptibility to another. For instance, there is emerging evidence that editing a gene to make an individual resistant to cystic fibrosis may increase vulnerability to severe influenza. Such unintended consequences underscore how much we still do not understand about gene interactions.

Moreover, many genetic conditions are not caused by a single gene mutation. The complexity increases substantially when more than one gene is involved, compounding the risk of unpredictable outcomes. The greater the number of edits required, the higher the uncertainty.

There is also a fundamental ethical divide that exists between therapeutic intervention and genetic enhancement. Most scientists and bioethicists agree that correcting a genetic mutation that causes a debilitating disease is ethically justifiable. However, editing genes for enhancement purposes, such as increasing intelligence or altering physical traits, is widely regarded as unethical. The challenge is that the boundary between therapy and enhancement is not always clear-cut. For example, is editing a genome to reduce susceptibility to common infections a therapeutic intervention or a form of enhancement?

The only known case of HHGE was reported in 2019, when a physician in China edited the embryos of three babies to confer resistance to HIV. From the limited data available, the outcomes have raised serious concerns. The edits were incomplete, resulting in mosaicism in one of the infants, where not all cells carry the same genetic edit, which introduces unknown and possibly harmful biological consequences. More importantly, these edits are now part of the children's germline, meaning they may be passed on to future generations, compounding the ethical and medical uncertainties.

In this study, the gene editing was not therapeutic in the traditional sense, as the children were not HIV-positive. It was a preventive measure that crossed into enhancement territory, reigniting ethical debates around the appropriate use of gene editing technologies.

This case is often cited as a cautionary example of why the global scientific community is not ready to proceed with HHGE. At recent meetings in Washington, DC, including collaborative discussions between ISCT, the Alliance for Regenerative Medicine (ARM), and the American Society of Gene and Cell Therapy (ASGCT), the consensus was clear—the

current moratorium on HHGE should be extended. Most countries with established regulatory frameworks already prohibit HHGE, and the scientific community broadly agrees that a minimum 10-year extension of the moratorium is necessary.

Beyond the ethical implications, there is also a practical question of whether HHGE offers a meaningful advantage compared to current or emerging therapies. Taking cystic fibrosis as an example, advances in supportive treatments and targeted therapies have significantly improved both life expectancy and quality of life for patients. As therapeutic options continue to evolve through small molecules and gene therapies, the justification for editing embryos becomes less compelling.

Additionally, if we reach the point where we can safely and effectively gene-edit a newborn with a monogenic disorder, the rationale for editing an embryo weakens further. We should not assume the unknown risks associated with modifying the germline when we can treat the condition at birth with known methods and outcomes.

Q Finally, looking ahead to the next 5 to 10 years in the gene editing field, what excites you most?

BJG What excites me most is the potential for these gene editing technologies to drive down COGs and, by extension, the cost of these therapies. Ultimately, it does not matter how many diseases we can cure if patients cannot access the treatments. That, to me, is the central issue. Scientific progress means little if it does not translate into availability and equity in care.

Currently, cost is a significant barrier. If I were a patient in need of treatment such as CAR-T cell therapy, I would have to seriously consider whether my insurance would cover it, whether I could afford the copay, and whether I could endure the treatment itself. This excludes the considerations of the logistical and emotional toll. While some of those questions are common to any serious illness, others are very specific to advanced therapies like gene transfer. That is where I believe we need to make meaningful progress.

One thing I found particularly compelling was a recent quote by Dr Peter Marks, where he stated that while we have made good strides with AAV-based therapies, we simply cannot afford to rely on them long-term due to their production costs. His view is that the field will need to shift more heavily toward gene editing technologies due to their economic viability. It is not about turning away from AAV, but getting to a place where therapies are not only effective, but also scalable and accessible.

It is incredibly rewarding to be part of this field at this point. We have seen enormous progress in less than 30 years. Patients have gone from being at a point in their treatment journey where they have no remaining options to achieving a complete remission following a treatment with a CGT product. We now not only have CGT products on the market, we have a growing pipeline of therapies, and expanding indications. This momentum is exciting; however, if we cannot find a way to get these treatments to more people, then the overall goal is incomplete.

My hope is that gene editing technologies such as CRISPR will not just expand scientific capabilities, but will be the key to making these therapies more broadly available. That is where the next big step forward lies.

BIOGRAPHY

For over 30 years, **Bambi Grilley** has worked primarily in the field of clinical research, focused predominately on oncology. She worked in and supervised the Investigational Drug Pharmacy at MD Anderson Cancer Center for 10 years and following that, she accepted a position as the Administrator of the IRB and IACUC at Baylor College of Medicine and served in that position for 2 years. For 25 years she served on the BCM IRB and for the majority of that time, as a vice-Chair. She is currently a Professor, Pediatrics at BCM and the Director of Clinical Research and Early Product Development for the Center for Cell and Gene Therapy where she is responsible for coordinating the development, implementation, and conduct of clinical research protocols for use in four affiliated hospitals and institutions. Her expertise has helped to establish the Protocol Review Committees, the Data Review Committees, the Clinical Research Quality Control Program and the Clinical Research Quality Assurance Program. In the 28 years she has been with CAGT, she has overseen 69 Research INDs covering 130 clinical trials, 52 additional INDs for patient specific access to investigational therapies, an Orphan Drug approval, and 2 Cost Recovery approvals.

Bambi is owner of QB Regulatory Consulting, LLC through which she has expanded her skillset to include support of several start-up companies, primarily in the cell and gene therapy space by providing regulatory affairs consulting and project management support. She has helped those start-up companies develop regulatory strategies, make regulatory submissions (including a company sponsored IND), and in some cases conduct clinical trials. She is a regular reviewer on the California Institute for Regenerative Medicine (CIRM) panel which awards grants to further clinical and translational work. Bambi is also a very active member of the International Society for Cell & Gene Therapy (ISCT), currently serving on its Board of Directors as Chief Regulatory Officer, Chair of Cross Border Working Group, and executive/leadership roles on several other of its committees. As CRO, Bambi has represented ISCT at a number of unprecedented collaborative events, two of which recently occurred in March and June of this year: the Guardrails on Heritable Human Genome Editing summit with ARM and ASGCT and the FDA Cell and Gene Therapy Roundtable which brought together the leaders of HHS, NIH, CMS, and CBER. She is a patient advocate and works tirelessly to address the 'valley of death' of academic GMCT products, most recently by joining the American Society for Transplantation and Cellular Therapy's (ASTCT) task force, ACT To Sustain (Adoptive Cell Therapy to Sustain), publishing a paper in *Transplant and Cellular Therapy* titled 'ACT To Sustain: Adoptive Cell Therapy To Sustain access to non-commercialized genetically modified cell therapies'. Her most recent publications can be found in this year's January and February online ahead of print issues of *Cytotherapy*—'Sickle cell disease gene therapy drug expenses and reimbursement: a litmus test for commercial pricing strategy and patient access for curative therapies' and 'International Society for Cell & Gene Therapy Expanded Access Working Group position paper: key considerations to support equitable and ethical expanded access to investigational cell- and gene-based interventions'.

Bambi is the recipient of The Norton Rose Fulbright Faculty Excellence Award and the 2021 AACR Team Science Award for her work with the St Baldrick's Foundation—Stand Up To Cancer Pediatric Cancer Dream Team.

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CELL & GENE THERAPY INSIGHTS

NEXT STEPS IN THE
GENE EDITING REVOLUTION

SPOTLIGHT

Genome editing: decoding the technical challenges and balancing precision, safety, and global regulations

Houria Bachtarzi

VIEWPOINT

"AI and machine learning will play an increasingly central role in research and development activities, from predicting the best targets for editing to enhancing precision and efficiency to reducing off-target effects."

Houria Bachtarzi is a gene and cell therapy consultant operating at the forefront of technical CMC, regulatory science, and strategic development. In an industry where no product or technology is ever the same, her role is to provide a tailored scientific and regulatory insight to address these complexities effectively. Here, Houria explores the current landscape and future direction of gene-edited therapies, with a focus on technical challenges, regulatory development, and emerging platform technologies. She examines the scientific and policy milestones shaping clinical success, long-term safety, and reaching the global stage where genetic medicines have no borders.

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THE TECHNICAL & REGULATORY PUZZLE OF THERAPEUTIC GENOME EDITING

One of the most significant technical and development challenges still facing the genome editing field is the sensitive detection of off-target gene editing effects. There remains a pressing need for standardized, highly sensitive, and specific tools capable of identifying off-target gene editing events, taking into account potential human genetic variations, which can add another layer of difficulty, as individuals may respond differently to gene-editing interventions. Hence the need for risk assessing the impact of potential variations within the target disease population, when considering treatment with gene editing.

Although a number of genome-wide analysis techniques are available to detect off-target and unintended on-target gene editing events, these methods require further standardization and validation to ensure consistent and reliable results. Another major challenge arises in the context of *in vivo* genome editing products, especially those intended for systemic administration. The potential risk of uptake into non-target tissues, including germline cells, presents an important safety concern, which warrants careful consideration. At present, there are no widely adopted, standardized methods for assessing whether germline modification has occurred in treated individuals. This is complicated by the lack of appropriate predictive animal models. This is a significant gap; and with more *in vivo* genome editing therapies entering the clinic, more efforts should be taken to develop highly sensitive, specific and biologically relevant tools to better asses the risk of germline transmission.

In the case of systemic administration, the issue of delivery remains paramount.

Delivery remains a significant bottleneck to achieving efficient and safe transfer of gene editing components *in vivo*. There is therefore a clear need for more robust viral and non-viral delivery systems that are suitable for systemic use and can efficiently deliver the therapeutic payload (the genome editing machinery), hence ensuring that sufficient amount of this payload can:

- Reach the distant target sites
- Achieve the desired level of editing at those sites
- Subsequently mediate the desired level of therapeutic activity

Having these delivery tools in hand will help expand and widen the therapeutic application of this technology beyond liver-targeted indications or those amenable to a local administration approach only.

From a regulatory standpoint, with genome editing technologies evolving rapidly, regulators face the challenge of balancing safety with the desire to speed up innovation. Considering the novelty and rapid evolution of this technology, it is not uncommon to encounter differing views on the technical and scientific requirements, as well as the extent of data needed to demonstrate product quality, safety, and efficacy. There is a need for ongoing dialogue and harmonization across different jurisdictions to support the continuous advancement of gene editing therapies.

IMPLEMENTING LONG-TERM MONITORING STRATEGIES

Demonstrating the long-term safety and durability of gene editing interventions requires a comprehensive and carefully designed follow-up strategy. Long-term safety is typically assessed through extended monitoring for delayed adverse events, including those arising from off-target effects, which may predispose

individuals to malignancies or other unpredictable outcomes that could manifest or only become apparent years after treatment. Current regulatory expectations call for up to a 15-year follow-up period for patients treated with gene editing therapies. This includes in-person clinical assessment including physical examination, laboratory tests and imaging, during the first five years, followed by annual monitoring through questionnaires and medical records' review. Should new safety signals or concerns arise, the duration and intensity of follow-up will need to be adjusted accordingly.

Molecular testing, particularly for off-target effects, should be integrated into the monitoring plan. The incorporation of such testing can be informed by findings and observations from preclinical development activities, including *in vivo*, *in vitro*, and *in silico* analyses. The level, nature and potential clinical relevance of those identified off-target effects, as well as the type of tissue/organ system being targeted, should guide the clinical evaluation strategy. For example, for liver-targeted gene editing interventions, preclinical evidence of off-target effects may necessitate monitoring for liver cancer in treated individuals.

In the case of systemically administered products, safety monitoring should also account for adverse events arising from potential off-target effects in non-target tissues and other susceptible organs.

Immunogenicity is another important consideration, which should take into account the immune status of the treated individuals, the type of product used including the nature of the gene editing components and the type of delivery system employed (viral versus non-viral vectors). Assessment of immunogenicity should be considered during non-clinical development, with additional monitoring implemented during clinical development. For *ex vivo* gene-edited cells, the potential risk of immunogenicity is mitigated through rigorous testing of the final cell product to demonstrate clearance of immunogenic components such as residual nucleases. However, attention must be paid to *in vivo* genome editing products. In these cases, a 'hit-and-run' approach using transiently expressed gene editing therapeutics, can offer a distinct advantage by limiting both the persistence and activity of nucleases, thereby reducing the risk of immunogenicity and off-target effects.

The monitoring plan for long-term durability of genomic modification and the resulting therapeutic response will depend on the indication being treated. Durability can be monitored using disease-specific biomarkers, which serve as 'surrogate durability' endpoints to assess the persistence and effectiveness of the therapeutic intervention over time.

EXPANDING THE GENOME EDITING TOOLBOX

Novel platform technologies and tools are revolutionizing the field of genome editing by enabling precise, efficient, and versatile gene modifications to be done with reduced off-target effects. First-generation modalities such as those based on clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9, transcription activator-like effector nucleases (TALENs), and zinc finger nucleases (ZFNs) remain powerful clinically validated tools. The impact of CRISPR/Cas9-based gene editing is already evident in the treatment of patients with hereditary hemoglobinopathies, including sickle cell disease and transfusion-dependent β-thalassemia.

However, for certain pathologies, newer technologies such as prime editing, base editing, or gene writing, may offer a more favorable benefit–risk profile. Both base editing and prime editing do not induce double-stranded DNA breaks, thereby reducing the risk of insertions/deletions, and chromosomal rearrangements. *In vivo*

base editing approaches utilizing lipid nanoparticle (LNP) delivery systems, are showing promising results in cardiovascular diseases, for example, targeting the *PCSK9* gene to lower LDL cholesterol as well as in metabolic disorders such as alpha-1 antitrypsin deficiency.

Base editing is also being explored for sickle cell disease and transfusion-dependent beta-thalassemia, where current therapies have predominantly been based on *ex vivo* approaches. Transitioning to an *in vivo* base editing approach could make these treatments more affordable and accessible globally, especially in low- and middle-income countries. The simplicity of LNP-based delivery systems has the potential to democratize access to gene-based therapies, reinforcing the vision that genetic medicine should have no borders.

Artificial intelligence (AI) is also beginning to have a profound impact on genome editing. Its integration into the field is facilitating prediction of optimal targets for editing, improving editing precision and efficiency, and helping to minimize off-target effects. AI can potentially enable the rapid analysis of vast genomic datasets, uncovering previously unnoticed patterns and facilitating the development of more powerful gene editing systems. For instance, machine learning tools such as Easy-Prime have successfully been used to optimize the design of prime editor guide RNAs (pegRNAs). Such knowledge-based trained tools were in fact shown to predict editing efficiency, hence facilitating prioritization of candidate pegRNAs.

Companies like Profluent Bio have demonstrated the potential of AI-driven gene editor design, by generating gene editors with comparable or improved activity and specificity compared to SpCas9. Their AI-designed OpenCRISPR-1 was also shown to be compatible with base editing.

These examples underscore the transformative role of AI and machine learning in advancing genome editing platforms.

EVOLVING CMC STANDARDS & REGULATORY FRAMEWORKS

The future of CMC along with broader regulatory guidance for genome editing will undergo continuous evolution, driven by the pace of scientific innovation, the emergence of diverse platform technologies, and the advancement of analytical methods that are needed to support them (notably those related to genome-wide analysis of off-target effects and safety methods for characterizing chromosomal abnormalities).

At present, developers can leverage a substantial body of existing regulatory guidance documents including those related to gene therapy and genetically modified cell-based products, as well as the FDA's guidance on human genome editing products. However, gaps remain, and further guidance will inevitably emerge as more clinical experience, particularly with *in vivo* genome editing products, is gained. The accumulation of real-world data will inform future regulatory expectations and help refine standards for safety and efficacy.

Within the realm of CRISPR-Cas9-based gene editing, the most advanced technology in the field, multiple analytical methods are currently being employed to analyze CRISPR-Cas9-mediated off-target effects. These include *in silico*, cell-free, cell-based, whole genome sequencing, and *in vivo* methods. Each method offers distinct advantages and limitations, and careful consideration must be given when selecting the appropriate testing strategy for a given application.

The British Pharmacopoeia Commission has initiated efforts to publish guidance focused on CRISPR-Cas9 off-target analysis. This represents an important step toward establishing best practices in the field.

For *in vivo* genome editing products, particularly those administered systemically, improved detection and risk assessment methodologies are essential. The

development of best practice guidance on relevant tools and methodologies to assess the risk of germline transmission/unintended germline editing would be a welcome advancement. In a field as dynamic as genome editing, future regulatory guidance must remain flexible, grounded in a risk-based and science-driven approach. This flexibility is crucial to sustaining innovation and investment, while ensuring that quality, safety, and efficacy remain at the forefront of therapeutic development.

Another particularly transformative development is the FDA's establishment of the Platform Technology Designation Program for drug development. This initiative allows, for instance, a defined platform, comprising a specific process, gene editing components, delivery system, equipment, and analytical assays, to be potentially applied across multiple products without compromising quality, manufacturing standards and safety. By doing so, such a platform has the potential to result in real significant efficiencies in the development, manufacturing and regulatory processes.

This designation serves as a powerful regulatory tool, streamlining development and trimming costs by reducing redundant testing and validation requirements (notably those related to analytical methods and process validation). In addition, certain non-clinical safety data may also be leveraged across products developed using the same platform.

As such, it offers significant efficiencies in manufacturing, regulatory review, and overall product development timelines, thereby facilitating faster patient access and broader application across multiple indications.

While no equivalent designation currently exists in Europe, the European Medicines Agency (EMA) does support the use of 'prior knowledge' acquired from products derived from the same platform technology and manufactured using the same platform-based process.

ACHIEVING GLOBAL REGULATORY HARMONIZATION

Harmonization efforts within the cell and gene therapy sector are actively progressing, and there has never been a more opportune moment for international dialogue on the technical requirements and standards for advanced therapy medicinal products (ATMPs). The International Council for Harmonization (ICH) has established a dedicated technical forum to address the specific harmonization needs of cell and gene therapy products. This initiative is initially focused on modalities that are considered sufficiently mature, based on accumulated clinical and commercial experience to date.

Included in this initial harmonization effort are *ex vivo* genetically modified immune cell-based products, such as autologous and allogeneic CAR-T therapies, as well as AAV-based gene therapy products. One would expect that the scope of this work will eventually be expanded in the future, to cover other cell and gene therapy modalities including *in vivo* genome editing technologies, as the field matures and more clinical and commercial experience is gained.

The wide participation of various international regulatory authorities to achieve this harmonization goal is notable. Regulatory authorities and associations from across the globe are actively involved, including representatives from North America (US FDA and Health Canada), South America (Brazil and Argentina), the MENA region (Algeria, Egypt, and Saudi Arabia), the UK (MHRA), the European Commission, Swissmedic, Japan's PMDA, and key Asian regulatory bodies from Singapore, China, and the Republic of Korea. The World Health Organization (WHO), the International Pharmaceutical Regulators Program (IPRP), the United States Pharmacopeia (USP), and the European Directorate for the Quality of Medicines

(EDQM) are also contributing, alongside other industry associations.

This collective effort marks a significant milestone, especially considering that many of the existing ICH guidelines were originally developed with conventional biologics, such as monoclonal antibodies, in mind. As a result, certain CMC recommendations within these guidelines are not readily applicable to ATMPs and do not take into account their unique features and challenges. As such, there is an urgent need for revised guidance that reflects the specific technical, scientific and regulatory considerations of these innovative therapies.

This international collaboration and harmonization will be essential to streamline the development, accessibility, and global adoption of advanced therapeutic technologies.

TOWARD A SMARTER, SAFER, MORE EQUITABLE ERA

Therapeutic genome editing has made remarkable progress in recent years. The marketing approval of the first *ex vivo* genome editing product has marked a major milestone, and it is anticipated that the first *in vivo* genome editing product will soon follow, with some candidates already in pivotal clinical trials. This will be a significant milestone for the entire field.

Over the next 5–10 years, second-generation gene editing technologies, such as prime editing and base editing, are expected to mature and advance toward late-stage development. As innovations in delivery systems continue, including LNPs, AI-engineered viral capsids with enhanced tissue specificity, and novel non-viral platforms, the therapeutic landscape will expand beyond liver-targeted diseases. These advances will enable broader application across a wider range of indications.

AI and machine learning will play an increasingly central role in research and development activities, from predicting the best targets for editing to enhancing precision and efficiency to reducing off-target effects. Regulatory frameworks will also evolve, with greater recognition of genome editing as a platform technology. This will facilitate streamlined approvals for therapies targeting different mutations using a shared core editing approach.

Globalization of clinical and commercial activities is expected to accelerate and expand beyond North America, Europe and Asia Pacific to other regions including the Middle East, Africa and South America. Looking ahead, several priorities must be addressed to ensure equitable and sustainable progress. Equitable access and affordability must be prioritized to ensure that gene editing therapies are available worldwide, not only in high-income countries. Robust safety and long-term monitoring efforts should continue, with continued focus on minimizing off-target effects and generating long-term safety data through real-world evidence and international registries.

There is also a need to develop highly sensitive, specific, and biologically relevant tools to better assess the risk of germline transmission. Regulatory innovation will be critical, including the adoption of adaptive, platform-based frameworks that can keep pace with scientific advances and enable faster, safer translation to patients. Integration with complementary technologies, particularly AI and machine learning, will continue to drive faster transformative advances in genome editing.

BIOGRAPHY

Houria Bachtarzi is the Principal Consultant of BIOCELLGENE Consulting Ltd, with strong expertise in the development and regulatory strategic aspects of advanced biological therapies covering virally vectored gene therapy, *in vivo* and *ex vivo* gene editing, genetically modified cells for tissue regeneration, genetically engineered immune cells targeting cancer cells, cell-based immunotherapy, stem cell-based therapies; as well as other innovative therapeutic modalities. She started her career by training and registering as a pharmacist in the UK; and completed her PhD in Cancer Gene Therapy at the Department of Oncology, University of Oxford, Oxford, UK. She subsequently followed up with additional post-doctoral work in AAV-based gene therapy and shRNA therapeutics for degenerative neuromuscular disorders at the Centre for Biomedical Sciences, Royal Holloway–University of London, London, UK. Prior to founding BIOCELLGENE Consulting Ltd, Houria was a Director of CMC Regulatory Affairs (Consultancy) at Precision for Medicine, acting as an internal and external subject matter expert, to support organizations developing CGT products and biologics for rare diseases and oncology indications. She also held senior positions at product development and regulatory consultancies, notably Associate Director of Regulatory Sciences/ATMP Subject Matter Expert at ProPharma Group and Senior Consultant positions at ERA Consulting (now Cencora PharmaLex), NDA Group and Dark Horse Consulting.

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AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

DOWNSTREAM PROCESSING

INNOVATOR INSIGHT

Proof of concept of a fully enclosed CAR-T process without use of a biosafety cabinet

Jon Pileggi, Menna Siddiqui, Carlos Ramos, Diana Santana, Christopher Alvarado, Brittany Miller, Cathy Wang, Forrest Kan, and Chantale Bernatchez

This proof-of-concept study evaluated the feasibility of using CellSeal Connect vials as closed-system alternatives to conventional cryovials, with the goal of eliminating reliance on biosafety cabinets (BSCs) during CAR-T manufacturing. Enriched T cells from healthy donors and lentiviral vectors were filled and cryopreserved in both CellSeal Connect vials and standard cryovials, then used in a CAR-T process. Additionally, BioLife Solutions' CellSeal® CryoCases, which are a rigid and transparent primary storage container, were evaluated as a closed system option for filling final drug product. Results demonstrated comparable cell expansion, viability, and transduction efficiency between cells cryopreserved in the CellSeal Connect vials and standard cryovials. Additionally, post-thaw final drug product was similar between cryovials and CellSeal CryoCases. These findings support the feasibility of eliminating BSC use in standard CAR-T manufacturing, which can potentially reduce contamination risk, facility complexity, and cost in CAR-T production.

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INTRODUCTION

CAR-T cell therapy has emerged as a transformative approach in cancer treatment, offering hope to patients with refractory disease. However, CAR-T manufacturing is often complex and labor-intensive, posing significant logistical and operational challenges [1]. While multiple automated systems are available for closing CAR-T processing, a major limitation of these systems is that they can only enable a truly end-to-end enclosed process if all materials are packaged appropriately [2,3]. Almost universally, cryopreserved starting cells and viral vectors are packaged in containers that require the use of a biosafety cabinet (BSC) to access and transfer their contents into weldable containers to avoid sterility breach. Although BSCs are

effective in maintaining aseptic conditions during open processing steps, this approach introduces other challenges related to spatial constraints and the need for increased environmental monitoring during manufacturing [4].

Typically, BSCs are used for several key processing steps, including compounding media and accessing thawed cells and thawed viral vectors. Solutions exist for compounding media without the use of a BSC, including sterile filtration (e.g., in-line weldable filters) and cytokines packaged in weldable containers. If thawed cells and viral vectors can also be accessed in a sterile manner, an entire CAR-T manufacturing process can be performed without requiring a BSC. This study aimed to demonstrate the feasibility of a fully enclosed CAR-T process by using BioLife Solutions' CellSeal® Connect vials, which allow for a fully closed, sterile pathway for not only filling of critical materials, including viral vectors and starting cells, but also for retrieval at time of use.

STUDY DESIGN

This study was designed to compare a traditional CAR-T manufacturing process using cryovials and BSCs with a fully closed system utilizing CellSeal Connect vials for containment of starting cells and viral vector and CellSeal CryoCases for containment of final drug product (Figure 1). The objective was to determine whether the closed-system process could produce similar results in terms of expansion and transduction compared to a standard process while eliminating the need for a BSC.

MATERIALS & METHODS

Lentiviral vector

Anti-CD19 CAR/GFP lentiviral vector was generated following standard CTMC protocols and filled into either 1.8 mL cryovials or 2 mL CellSeal Connect vials using a BioLife Solutions' Signata CT-5™ fluid handling platform. Following fill, vials were stored at ≤-80 °C until time of use.

Starting cells

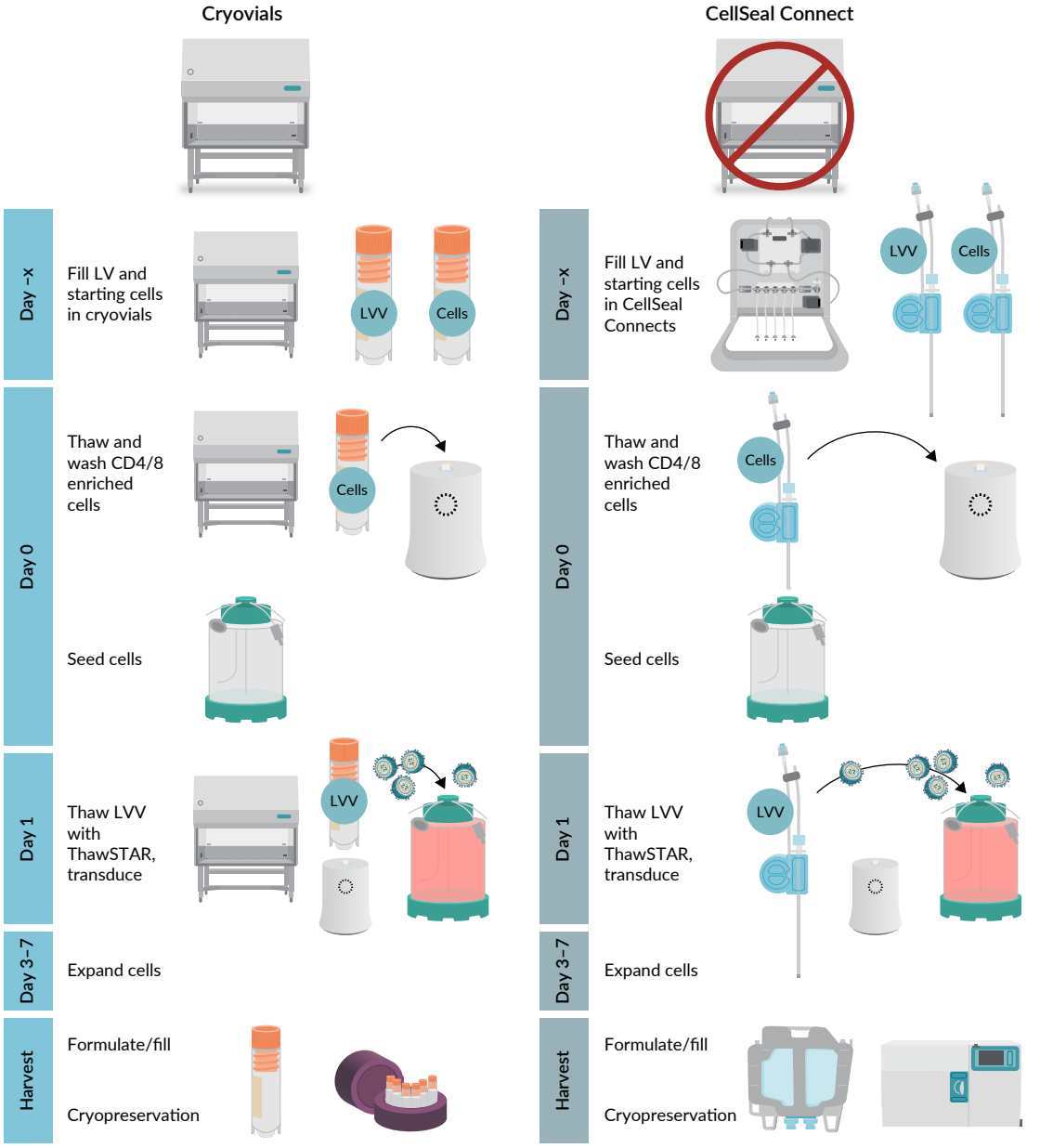
Three healthy donor leukapheresis collections were procured from Gulf Coast Regional Blood Center and processed fresh. T cells were positively selected with $CD4^+/CD8^+$ paramagnetic beads using an enclosed and automated system. Following enrichment, isolated cells were formulated into cryopreservation solution and then filled into either 1.8 mL cryovials, or 2 mL CellSeal Connect vials. Cells were then cryopreserved using a controlled rate freezer and stored in cryogenic freezers.

Thawing, activation, transduction, and expansion of cells

Containers were thawed using an automated, water-free tool, BioLife Solutions' ThawSTAR® thawing system. A ThawSTAR® CFT was used for cryovials, and a ThawSTAR® CSV was used for CellSeal Connect vials. Vials were inserted into each ThawSTAR unit and automatically released upon completion of thawing (~2 minutes, 30 seconds). Cells were then washed, resuspended in culture media, and activated using STEMCELL Technologies' ImmunoCult™ Human CD3/CD28/CD2 T Cell activator in G-Rex flasks. The day after activation, each culture was transduced using the aCD19 CAR/GFP Lentiviral Vector (LVV). For cultures initiated with T cells cryopreserved in CellSeal Connect vials, LVV filled in CellSeal Connect vials was used. For cultures initiated using T cells in cryovials, LVV from 1.8 mL cryovials were used. LVV was thawed using the ThawSTAR system and added at an MOI of 1.9, followed by a 48 hour incubation. Cultures were then fed with complete media and incubated an additional 96 hours.

FIGURE 1

Each workflow was executed in parallel using cells from three healthy donors.



Aside from the enclosures for starting cells and viral vector, all conditions were kept the same for each process. Following expansion, cells were cryopreserved in either cryovials or CellSeal CryoCases.

Harvest, formulation, and cryopreservation

At the end of expansion, each culture was harvested and washed into cryopreservation solution. Formulated cells were filled into either cryovials or CellSeal CryoCases. Cryovials were cryopreserved using Corning® CoolCell® containers in a -80°C freezer, while CellSeal CryoCases

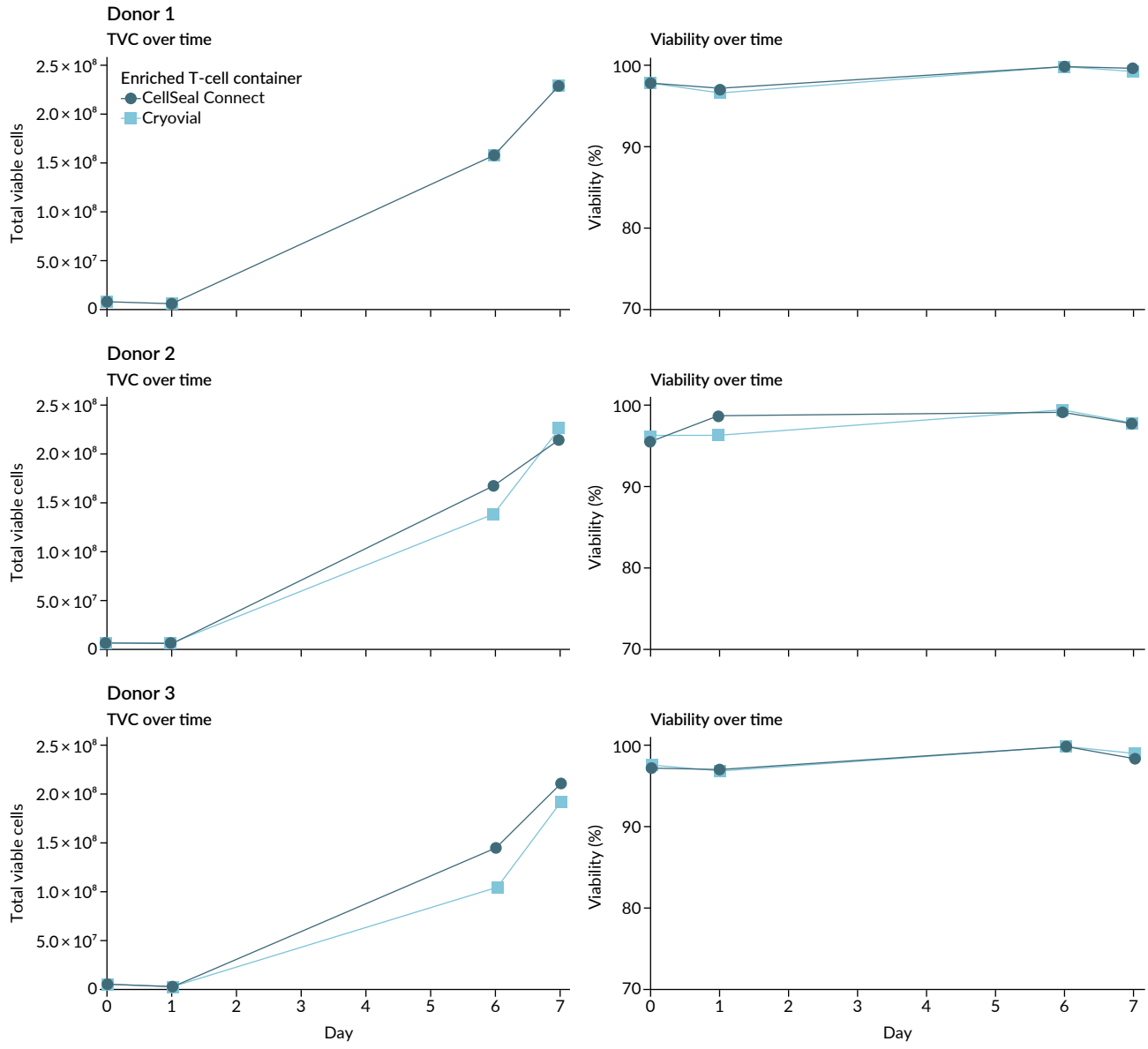
were cryopreserved using a controlled rate freezer. All samples were stored in cryogenic freezers post-cryopreservation.

Post thaw testing

CellSeal CryoCases and cryovials were thawed using a water bath. Cell count and viability were measured post thaw. 1.5×10^6 cells from each donor and container were washed into complete medium and seeded into 6 well plates at a concentration of 5×10^5 viable cells/mL for further culture. Cells were counted using a ChemoMetec NucleoCounter® NC-200™ at 24 and 48 hours post thaw.

FIGURE 2

In process cell counts and viability.



Across all donors, cultures initiated with enriched T cells cryopreserved in either CellSeal Connect vials or standard cryovials showed similar performance. Cell counts and viability were assessed using NucleoCounter NC-200.

Transduction testing

Transduction was measured by flow cytometry using a Cytek Aurora following standard CTMC protocols.

RESULTS

Expansion, viability, and transduction rate is similar for cells cryopreserved in CellSeal Connect vials or standard cryovials

Expansion kinetics were similar for cultures initiated with T cells cryopreserved in both CellSeal Connect vials and standard cryovials across all donors (Figure 2). Viability was high for all time points measured across all donors and conditions.

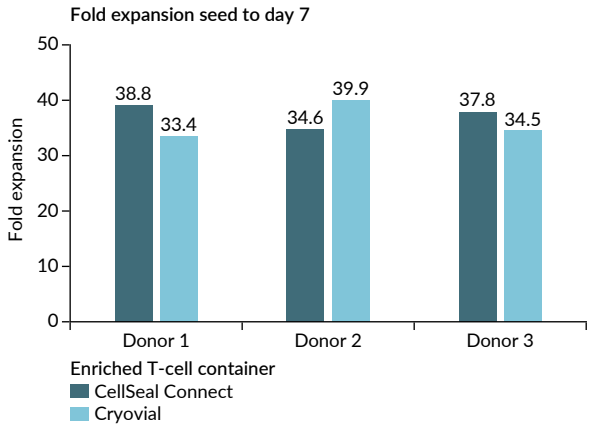
Fold expansion from seed to day 7 was consistently high across all donors and container types (Figure 3). Cultures initiated with cells cryopreserved in CellSeal Connect vials had expansions of 38.8×, 34.6×, and 37.8× for Donors 1, 2, and 3, respectively, compared to 33.4×, 39.9×, and 34.5× for cultures initiated with cells cryopreserved in cryovials. These results suggest no negative impact with the use of CellSeal Connect vials.

Transduction efficiency (Figure 4), measured as the percentage of CAR^+ (GFP^+) viable cells, was comparable between conditions. For cultures initiated with cells cryopreserved in CellSeal Connect vials, transduction rates were 43%, 54%, and 43% for Donors 1, 2, and 3, respectively. Cultures initiated with cells cryopreserved in cryovials yielded 49%, 59%, and 46% in the same donors. While some inter-donor variability was observed, the relative differences between container types were minor.

The number of CAR^+ cells generated through day 7 had some variability across donors but was similar regardless of the container used for cryopreservation of starting cells. Cultures initiated with cells cryopreserved in CellSeal Connect vials yielded 9.8×10^7, 1.2×10^8, and 9.1×10^7 CAR^+ cells for Donors 1–3, respectively. Cultures initiated with cells cryopreserved in cryovials yielded 1.1×10^8, 1.3×10^8, and 8.9×10^7 CAR^+ cells (Figure 5).

FIGURE 3

Fold expansion from seeding to day 7 was similar between conditions and across donors.



Total fold expansion was calculated by dividing the number of cells harvested on day 7 into the number of cells seeded on day 0.

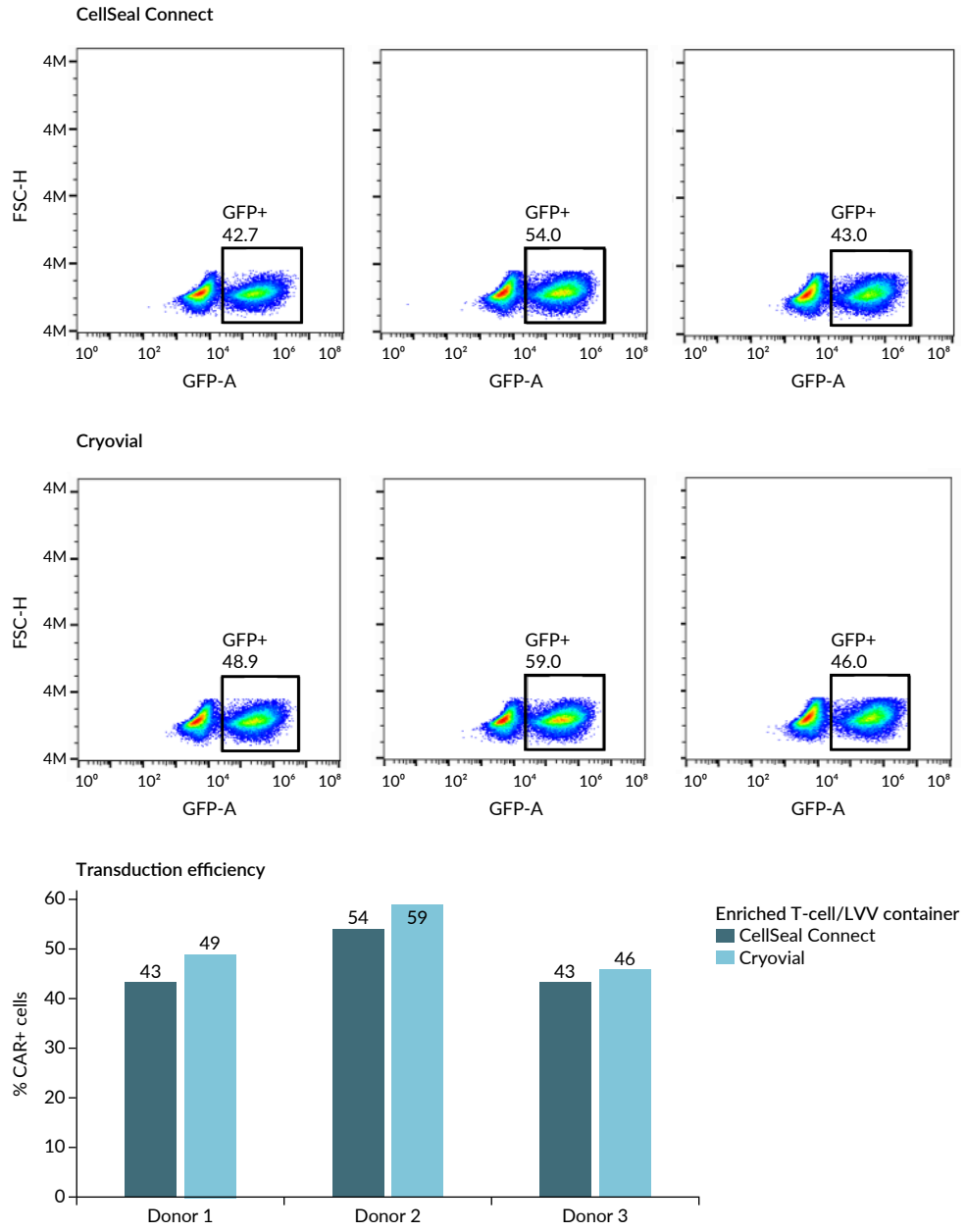
Post-thaw viability and recovery over 2 days is similar for final products expanded from cells cryopreserved in CellSeal Connect vials or standard cryovials

Immediate post thaw viability was high for final drug product cells cryopreserved in both CellSeal CryoCases and cryovials (Figure 6) across all donors. CellSeal CryoCase results ranged from 94.8% to 96.9%, while cryovials ranged from 97.1% to 97.5%.

Post rest viability was high at each timepoint measured across donors and conditions. Post rest cell recovery was measured by dividing the number of viable cells counted at 24 and 48 hours post rest into the total number of viable cells seeded into culture post thaw. Post rest recovery was high across donors and conditions with 2 of 3 donors achieving higher post rest

FIGURE 4

Transduction efficiency was variable across donors (D1, D2, D3) but similar across conditions.



Transduction efficiency was measured using a Cytek Aurora with CAR+ cells determined by the percentage of live single cells being GFP+. LVV: lentiviral vector.

expansion with cells cryopreserved using CellSeal CryoCases.

CONCLUSIONS

This study demonstrates that CAR-T manufacturing can be fully enclosed when appropriate containers are used for both starting cells and viral vectors. CellSeal Connect vials provide a functionally equivalent alternative to conventional screw cap cryovials, with comparable cell viability, expansion, and transduction efficiency (due to the small sample size, statistical analysis

was not performed). Additionally, CellSeal CryoCases can be used as a closed-system option for filling of final drug product. By enabling sterile welding for the extraction of thawed starting cells and viral vectors, the need for biosafety cabinets during CAR-T manufacturing can be eliminated. Taken together, these innovations reduce contamination risks, streamline operations, and simplify facility design.

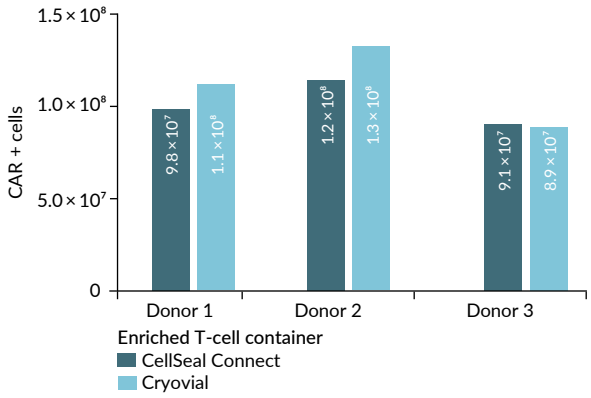
DISCUSSION

Although many manufacturing platforms are marketed as closed-system solutions for CAR-T manufacturing, all of these systems require an ancillary BSC if materials, primarily cryopreserved starting cells and viral vectors, are packaged in containers that do not support product extraction via sterile docking. The use of a BSC requires manual cleaning and adherence to aseptic techniques to ensure microbial-free culture. The front opening of the BSC used for transferring equipment and materials presents similar cleaning limitations and can disrupt air flow, increasing contamination risk. Additionally, BSCs have a large footprint, increase operating costs, and limit the number of subsequent operations due to the decontamination procedures required. In some instances, the use of a BSC may require special accommodation to the facility and is vulnerable to failures in the facility's infrastructure. Elimination of a BSC from CAR-T manufacturing can help simplify operations, reduce costs, and ultimately improve patient access to these therapies. CellSeal Connect Vials are a feasible option that can allow for both filling and retrieval of viral vector or starting cells for a CAR-T process without entering a BSC.

All BioLife primary containers used in this study, including CellSeal Connect Vials and CellSeal CryoCases, have undergone extractables and leachables testing and container closure integrity validation to meet CGT manufacturing requirements.

FIGURE 5

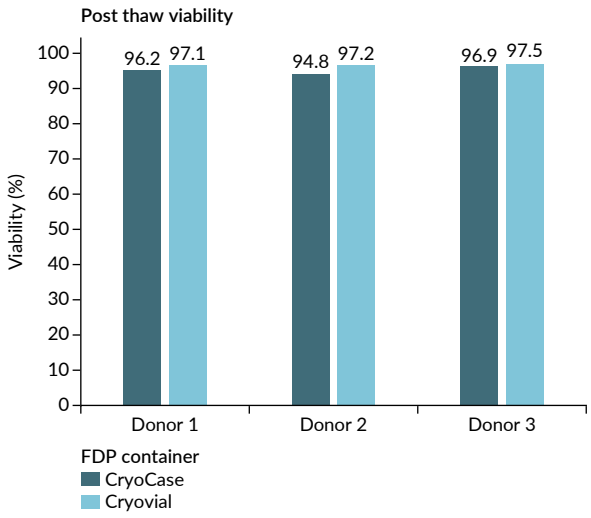
The total number of CAR$^+$ cells produced at harvest on day 7 was variable across donors but similar whether CellSeal Connect vials or cryovials were used.



The total number of CAR$^+$ cells was calculated by multiplying the percentage of transduced (GFP$^+$) cells by the total number of viable cells at harvest.

FIGURE 6

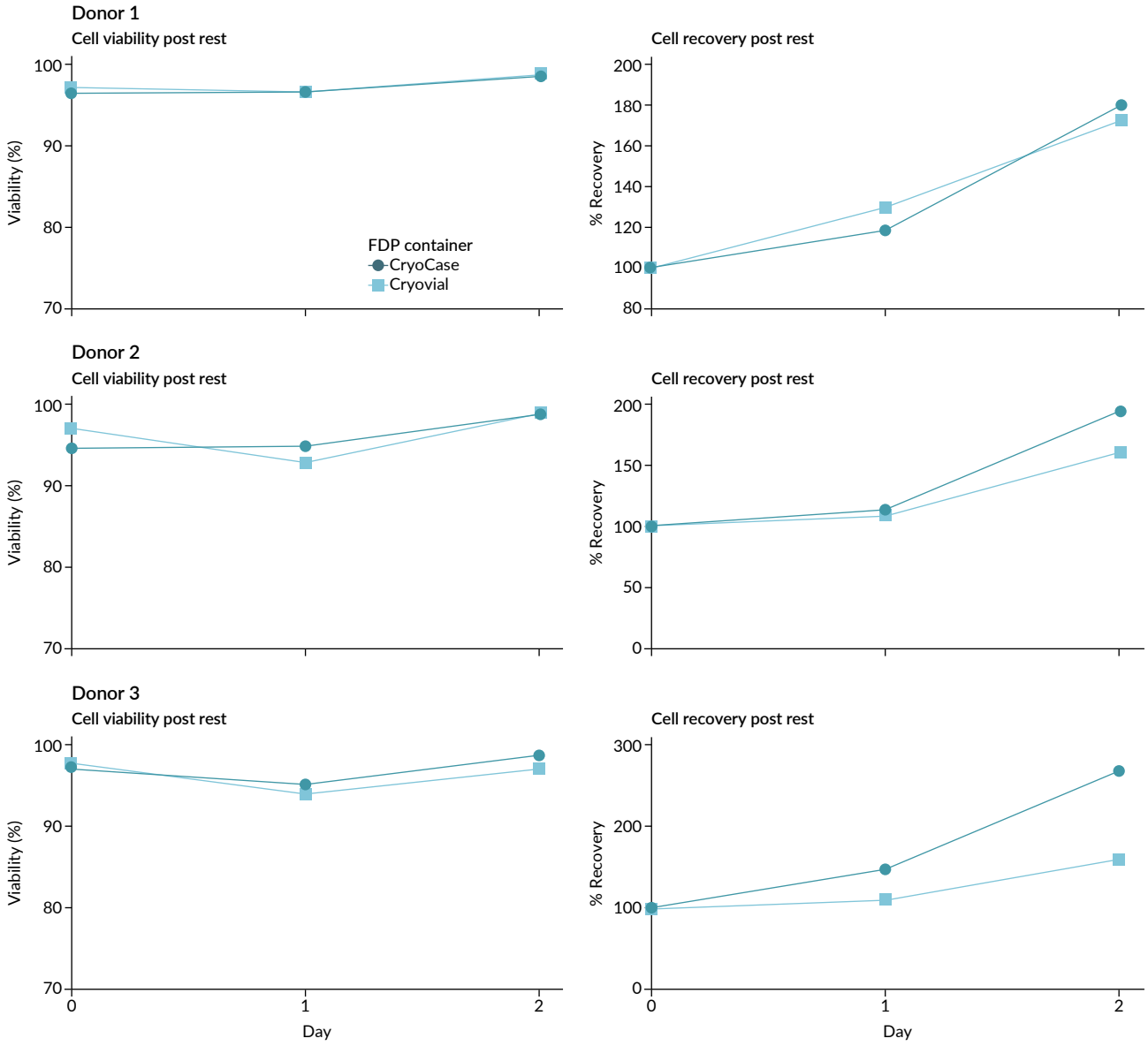
Post thaw viability was high across conditions and donors from both CellSeal CryoCases and cryovials.



These tests confirmed no reaction from leachable constituents, which is considered baseline qualification for all primary containers in clinical and commercial cell therapy processes. The data is available from the manufacturer to support evaluation and regulatory submissions.

FIGURE 7

Post rest cell viability was high across all donors, conditions and timepoints.



Post-rest cell recovery was as good or better with cells cryopreserved in CellSeal CryoCases compared to cryovials. Post rest cell recovery was measured by dividing the number of viable cells counted at 24 and 48 hours post rest into the total number of viable cells seeded into culture post thaw.

It is noted that the CellSeal Connect Vials have not yet been validated for storage at temperatures below -80°C as the weldable lines are composed of PVC, which can become fragile at cryogenic temperatures. During the execution of this study, no damage or deformity was noted as a result of storing vials at liquid nitrogen temperatures, however, additional testing may be needed to better assess the impact of cryogenic storage on container integrity. Proper handling and storage of CellSeal Connect vials to prevent fracture of these lines at time of use following cryopreservation would be critical.

One limitation of the CellSeal Connect vials is the volume capacity, as vials can

only hold a maximum of 5 mL of solution. In the context of standard autologous CAR-T manufacturing, these volume limitations need to be considered for both containment of cryopreserved starting cells and viral vectors. For many programs, $1–2 \times 10^8$ cells are needed for initiation of a manufacturing batches and if starting cells are cryopreserved at concentrations at or above 2×10^7 viable cells/mL, then only 1–2 CellSeal Connect vials would be needed per manufacturing run. For lentiviral vector containment, the volume limitations of the CellSeal Connect vials are not thought to be problematic as most programs use considerably less than 5 mL of LVV for transduction. However, this could be restrictive for programs using retrovirus depending on the volumes needed for transduction, which are routinely above 5 mL and can sometimes exceed 50 mL for transducing under 1×10^8 cells. For closed system filling and retrieval of retroviral vectors, it may be more practical to use weldable bags to reduce the number of containers needed.

In summary, while the CellSeal Connect vials can be used to further reduce the need for a BSC in CAR-T manufacturing, there are limitations that should be considered and evaluated to ensure their suitability for specific programs. While the volume capacities of the vials are well within the ranges of what are typically used for starting cells and lentiviral vectors, CellSeal Connect vials may not be well suited for retroviral vectors. Additional studies assessing the impact of cryogenic storage on container integrity would also be needed prior to integration of the vials into a program for use as a container for starting cells. Nevertheless, the use of CellSeal Connect vials represents an important step toward enabling a fully closed, BSC-independent CAR-T manufacturing process, which could ultimately improve scalability, reduce manufacturing costs, and expand patient access to these life-saving therapies.

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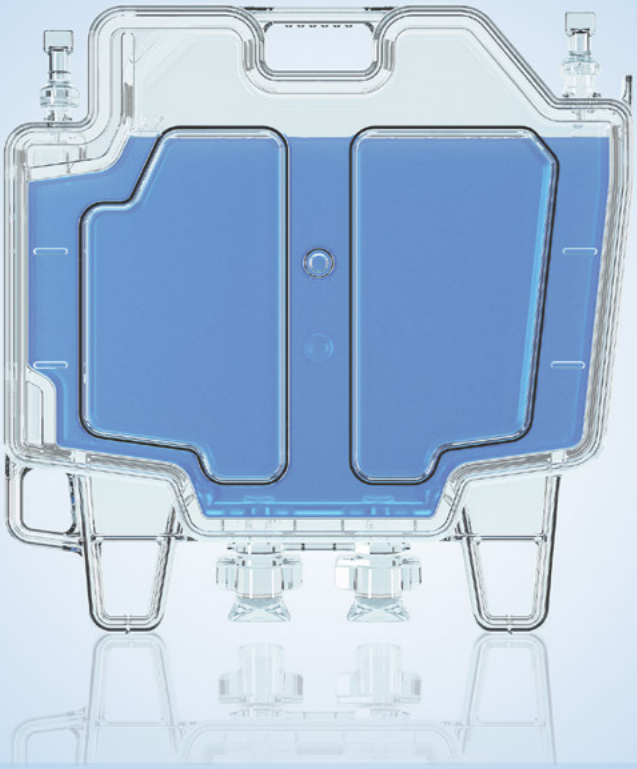

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DOWNSTREAM PROCESSING

The AAV CDMO market in August 2025: navigating the crossroads of capacity, complexity, and cost

Rahul Kaushik

VIEWPOINT

"In 2025, the real differentiator in AAV development isn't bioreactor capacity, it's rather forging risk-aligned partnerships where CDMOs and developers share both the milestones and the stakes."

In August 2025, the AAV CDMO market stands at a decisive turning point. Capacity is no longer the limiting factor; the real constraint is the scarcity of specialized expertise to navigate upstream scalability, downstream purity challenges, and intensifying regulatory scrutiny. Our analysis reveals a shift toward risk-sharing partnerships, where CDMOs act as both strategic investors and service providers, accelerating programs with minimal capital. Big pharma's pivot to 'platform-first' acquisitions further raises the stakes. For both developers and CDMOs, success now hinges on precise strategic alignment, deep disease-area specialization, and the ability to execute with speed, quality, and shared commitment.

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CURRENT REALITY

Our latest market intelligence indicates that the familiar narrative of 'capacity shortage' in AAV manufacturing is now outdated. As of August 2025, global GMP capacity for AAV production: upstream bioreactors, downstream purification lines, and QC suites are under-utilized in many facilities, running at an estimated 45–55% utilization compared to over 85 % in 2021.

The root challenge for developers has shifted. It is no longer about finding an empty slot on a 2000 L bioreactor schedule. The true bottleneck lies in accessing specialized expertise that can integrate upstream yield optimization, serotype-specific downstream purification, and regulatory-compliant analytical control into a coherent CMC strategy.

Our internal benchmarking shows that AAV developers who invest in strategic outsourcing preparedness: defining a robust target product profile (TPP), mapping the vendor landscape early, and aligning CMC choices with clinical/commercial endpoints are achieving regulatory submission milestones up to 8–12 months faster than those who approach outsourcing reactively.

CMC TRILEMMA: WHERE MOST AAV PROGRAMS STALL

Upstream processing: the scalability–speed trade-off

Despite years of process innovation, over 70% of AAV programs entering Phase 1 in 2025 still rely on transient triple transfection of suspension HEK293 cells. This platform offers speed for early toxicology material but introduces significant plasmid DNA supply chain risk. Our interviews with multiple suppliers confirm lead times for GMP plasmids now average 16–20 weeks, with cost per gram up 25–30% YoY. Stable producer cell lines, baculovirus–Sf9 systems, plant based AAV production systems, one plasmid systems etc. promise lower long-term COGS and simplified scale-up, but each carries regulatory familiarity gaps.

The trade-off is strategic: transient transfection offers rapid early entry into the clinic but locks in higher per-dose cost and complex plasmid logistics; stable systems reduce COGS but demand significant up-front development, cell bank characterization, and regulatory engagement.

Downstream processing: the pursuit of purity and yield

Downstream purification remains the most persistent technical hurdle. The separation of full from empty capsids, is not only a product quality attribute but a cost driver. Low full/empty ratios inflate dose volumes, increase vector demand, and can trigger additional safety concerns.

Affinity chromatography (e.g., AVB ligands, AAV-X) remains the dominant capture step, but without optimized polishing ion-exchange chromatography, density gradient ultracentrifugation, or novel ligand chemistries, many processes plateau at 30–40% overall recovery with suboptimal full/empty ratios.

These gains are rarely 'plug-and-play' for new clients. They require early alignment on serotype, formulation conditions, and in-process controls, plus investment in process characterization studies, often the first casualty when developers are pressed for time or funding.

Analytical development: when the assay defines the product

In 2025, regulatory agencies are applying unprecedented scrutiny to analytical control strategies. Phase-appropriate potency assays that truly reflect the vector's mechanism of action are now a focal point of IND and CTA reviews. We have tracked at least five clinical hold events in the past

18 months directly attributable to potency assay deficiencies.

The analytical toolbox is evolving. Basic qPCR for vector genomes and ELISA for capsid protein remain in use, but advanced techniques for particle heterogeneity and genome integrity are becoming mainstream. Multiplexed ddPCR to confirm the full length genome packaging, Capsid post translational modification analysis, charge detection mass spectrometry (CDMS), SecMALS are becoming mainstream in CDMO offerings.

A key risk we monitor is assay development lagging behind manufacturing scale-up. When analytics are outsourced to a separate CRO without close integration, timelines often slip. CDMOs with in-house analytical method development, qualification, and validation tightly coupled to upstream/downstream activities are increasingly preferred by sponsors.

FOR AAV DEVELOPERS: AVOIDING THE PITFALLS OF UNPREPAREDNESS

Our project post-mortem analyses identify three recurring mistakes that compromise outsourcing success:

- **Treating CDMOs as transactional vendors.** This mindset prevents the formation of integrated project teams and erodes accountability on both sides
- **Approaching CDMOs with commercially unviable processes.** Processes optimized for small-scale academic proof-of-concept often fail to translate to GMP, leading to costly re-engineering
- **Operating without a clear TPP.** Without a defined dose, delivery route, and target patient population, CMC decisions become reactive, causing avoidable rework

Developers entering the market in 2025 face a more discerning vendor landscape. CDMOs are selectively allocating scarce high-skilled resources to clients whose programs are both technically compelling and operationally ready.

FOR CDMOs: ATTRACTING AND RETAINING HIGH-VALUE PARTNERS

From the CDMO side, the challenge is reversed. Conversations with senior BD leaders confirm that under-prepared clients consume disproportionate resources, disrupt production schedules, and heighten regulatory risk. The most competitive CDMOs in 2025 are segmenting their client base and offering premium engagement models to well-capitalized, de-risked programs, while structuring milestone-based contracts for higher-risk sponsors.

Selection criteria are shifting. Developers increasingly rank project management discipline, communication transparency, and regulatory track record alongside technical capability. CDMOs able to act as a functional extension of the sponsor team, integrating manufacturing with regulatory and clinical considerations, are winning repeat business even in a slow funding environment.

STRATEGIC SHIFTS RESHAPING THE MARKET

The drug developers surviving these challenging market conditions are those who proactively invested in mapping out their critical path. They reverse-engineered their processes, workflows, and technologies to align them with their clinical reality. Conversely, developers who are currently in tough spots are the ones that burned through capital over the last couple of years just to reach the clinic. They are now realizing that clinical realities, investor scrutiny, and the interests of big pharma have shifted. Such developers are facing challenges with their

supposedly 'de-risked' clinical assets. It has become very evident that these programs have low viability for commercial success due to high Costs of Goods Sold (COGS), a stringent path to commercialization, and a lower-than-expected market acceptance rate. As a result, these clinical-stage programs are, in fact, not assets but liabilities.

Risk-sharing partnerships as a growth model

The most capital-efficient AAV programs reaching clinical stage in 2025, often with <20 employees and <$50 million total capital raised, share a defining trait: their CDMO partners were not just service providers, but strategic investors. In these arrangements, the CDMO's equity or milestone-based stake aligned incentives to accelerate the drug's path to clinic and beyond, with minimal capital outlay from the developer. We expect these risk-sharing structures to expand, as they provide CDMOs with upside potential and developers with critical execution capacity.

Big pharma's platform-first approach

Another notable shift is in acquisition strategy. Large pharma no longer prioritizes single-asset buys; instead, they are targeting platform capabilities, particularly in CNS delivery (e.g., Novartis/Voyager, Astellas/AviadoBio, Novartis/KateRx, Astellas/Sangamo, Eli Lilly/Sangamo). Deal structures are evolving to a 'try-before-you-buy' model with modest upfront payments tied to technology validation, followed by larger milestone payouts upon clinical proof-of-concept. This environment puts a premium on platform-oriented CDMOs that can demonstrate disease- or tissue-specific AAV capabilities.

Outlook for 2026

For single-pipeline companies, the priority is clear: secure a CDMO partner with true skin in the game and a willingness to share risk. For CDMOs, the imperative is to specialize not merely in generic AAV manufacturing, but in verticals such as cardiac, muscle, or brain-targeted therapies, coupled with deep disease-area and regulatory insight. This specialization creates leverage: capabilities honed for one client can be applied to another without reinvention, improving both technical efficiency and commercial appeal.

BIOGRAPHY

Rahul Kaushik is the Founder and CEO of **Gene Therapy Consultancy**, an AI-native firm dedicated to advancing AAV-based treatments for human diseases. His firm combines curated proprietary industry data and expert validation with advanced AI tools to deliver precise, up-to-date insights. This approach goes far beyond what generic AI systems or human experts can deliver alone.

The consultancy systematically removes hurdles for stakeholders across the gene therapy landscape—including founders, CEOs, investors, CDMOs, CROs, and technology developers. It does so by providing deep market insights and comprehensive technical, strategic, and regulatory due diligence. This process reduces drug development costs and risks, thereby fulfilling the firm's promise: '**Expert Insights, Smarter Decisions, and Faster Therapies**'.

With a career spanning a clinical-stage biotech in Munich, an innovative firm in Oxford, and a large global biotech, Rahul has cultivated deep expertise in solving critical gene

therapy challenges. His work focuses on strategic vector design, lead selection, and process development for CNS, metabolic, and ocular diseases.

As a seasoned scientific leader with over 16 years of R&D experience, he has established cutting-edge rAAV and LVV platforms, personally overseen the creation of more than 150 different rAAV vectors of various serotypes, and published over 25 papers in high-impact journals, including *Cell*, *Science*, *EMBO*, and *Nature Communications*.

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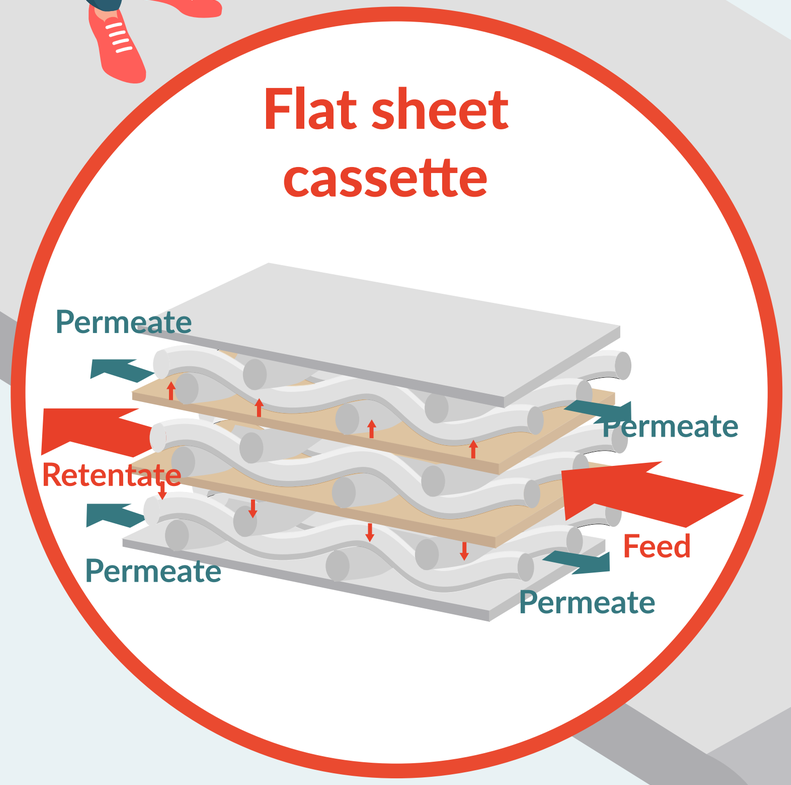
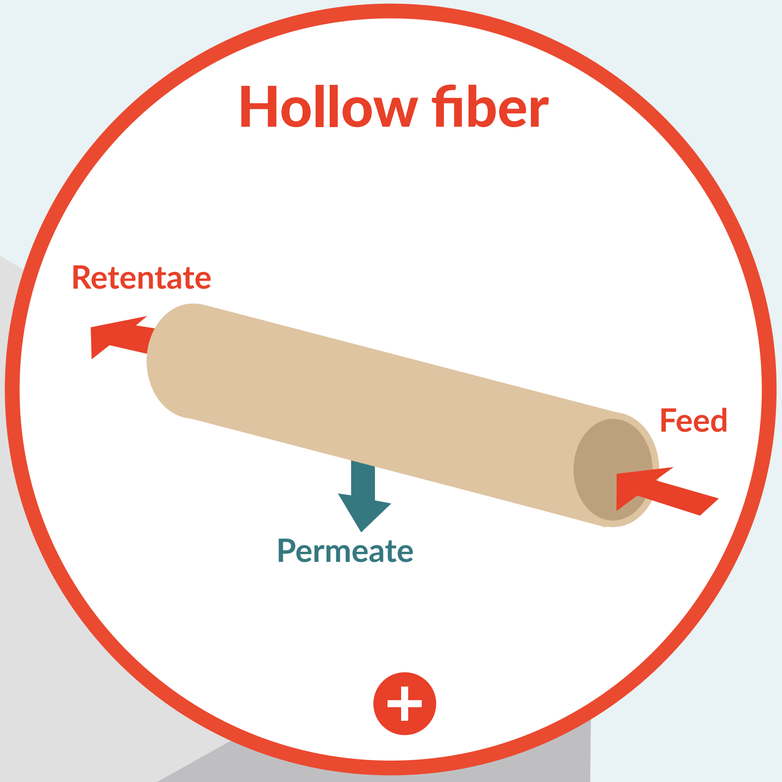
Optimizing your TFF process for AAV manufacturing:

a guide to evaluating hollow fibers versus flat sheets

Meet the speaker

Luke McCarney
MS in Chemical Engineering
from the University of Rochester





Hollow fibers versus flat sheets

A typical adeno-associated virus (AAV) manufacturing process involves two tangential flow filtration (TFF) steps: the pre-affinity step, which occurs toward the beginning of the process, and the final formulation step, which takes place toward the end.

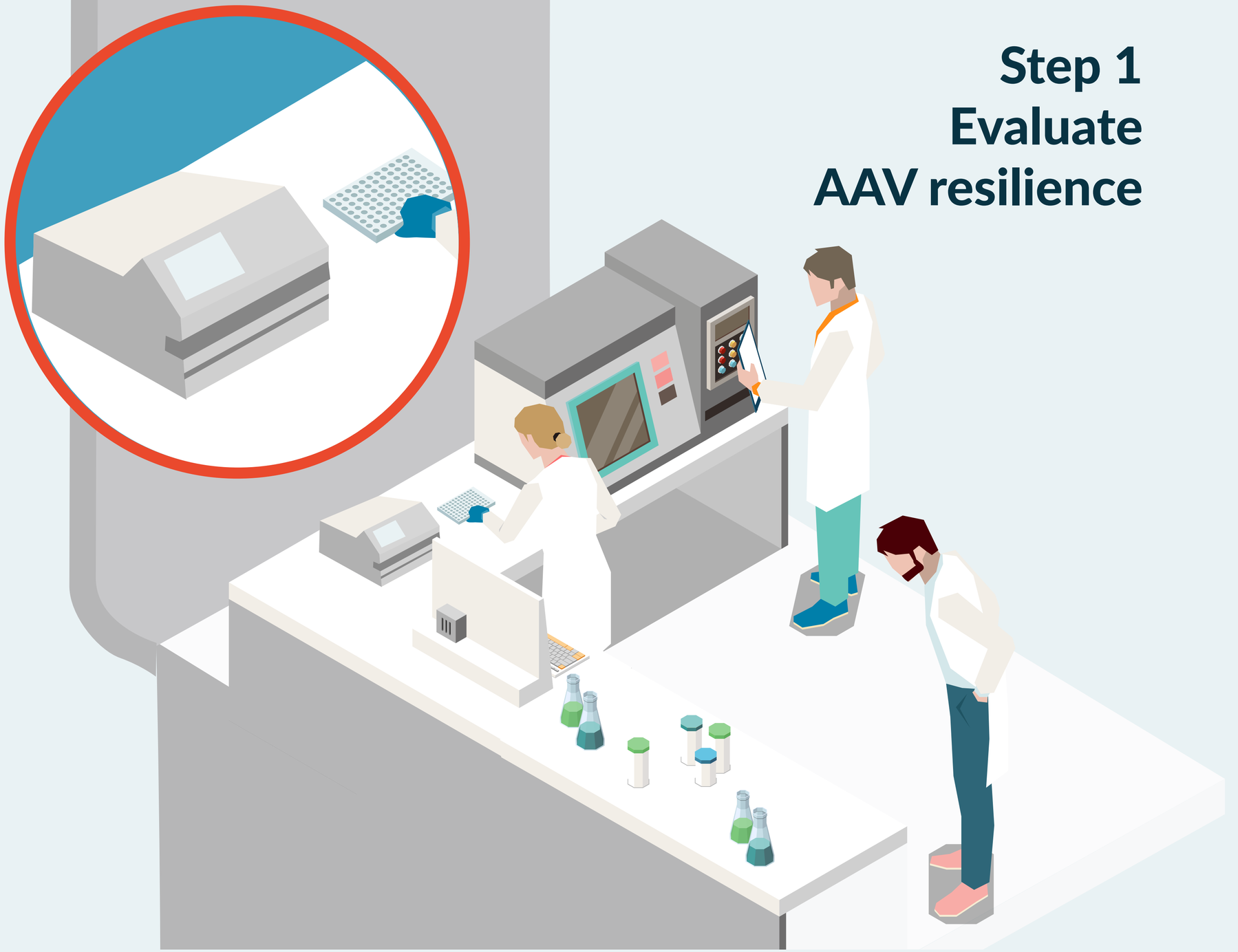
In bioprocessing, two commonly used TFF formats are hollow fibers and flat sheets. They both are utilized to concentrate or exchange the buffer of a product of interest.

Hollow fibers have unobstructed feed channels, typically resulting in laminar flow and lower shear, while flat sheets use screens that encourage turbulent flow, disturbing the concentration polarization layer and often enabling higher flux and shear rates.

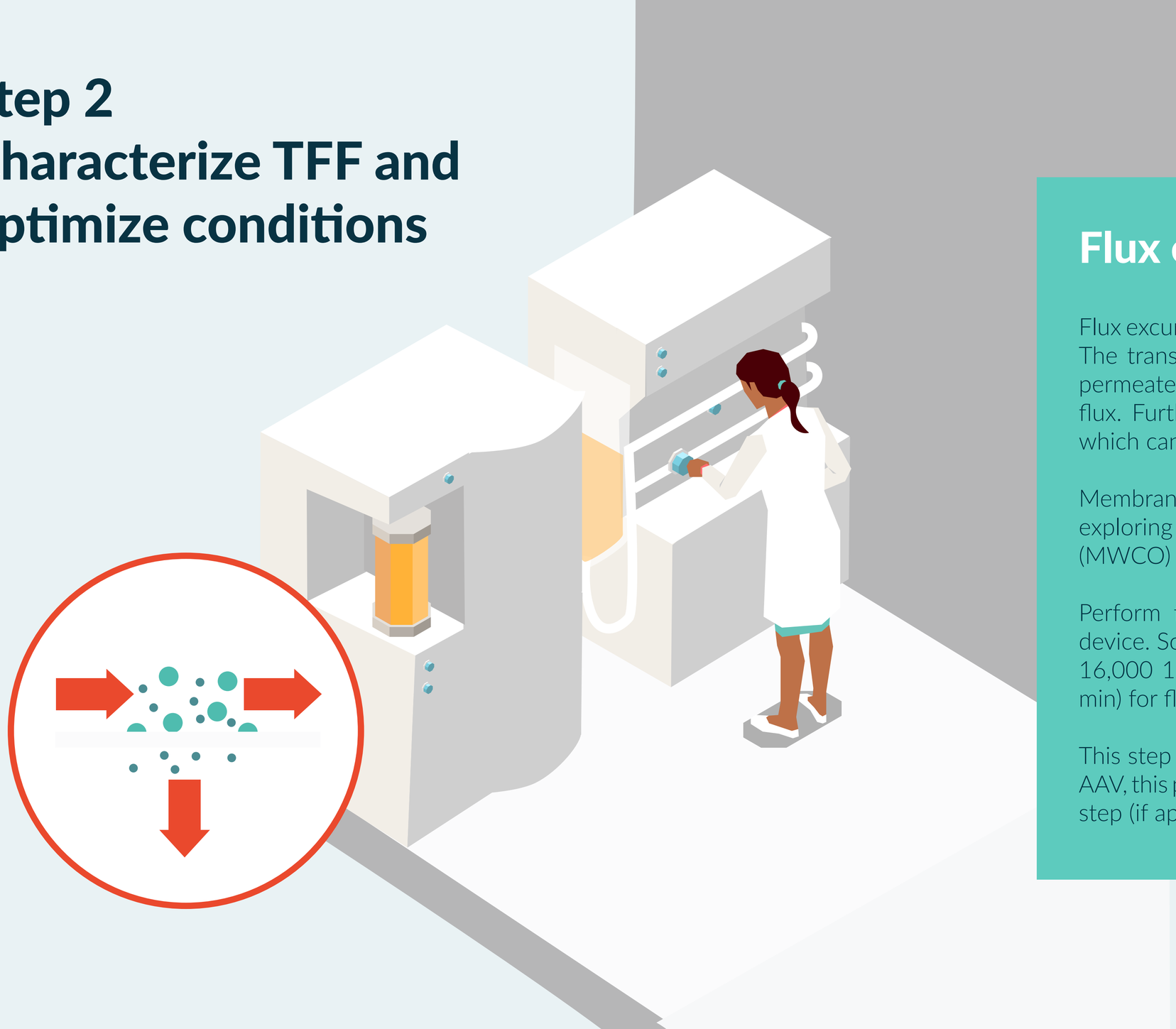
Step 1
Evaluate AAV resilience

Shear sensitivity is less well defined with novel modalities such as AAV. Therefore, it is important to evaluate target molecule resiliency within the TFF process. This evaluation can help define operational limits and may even provide evidence pointing toward one TFF format or another.

Evaluating resilience through each component of the TFF system (pumps, tubing, etc.) and then the TFF system as a whole is suggested to pinpoint system limitations. Evaluate critical quality attributes (CQAs), such as AAV titer, aggregation, etc., before and after recirculation to determine operational limitations.



Step 2
Characterize TFF and optimize conditions



Flux excursions

Flux excursions are used to optimize process conditions. The transmembrane pressure (TMP) is increased until permeate flux levels off, providing maximum permeate flux. Furthermore, different feed flows are evaluated, which can further optimize permeate flux.

Membrane selection can also be optimized at this step, exploring parameters such as molecular weight cut-off (MWCO) or chemistry.

Perform flux excursions varying flow rates for each device. Some common feed flows are 2000, 8000, and 16,000 1/s for hollow fibers, and 3, 5, and 7 L/(m^2 × min) for flat sheets.

This step should be repeated for all feed materials. For AAV, this process should be performed at the pre-affinity step (if applicable) and the final formulation step.

Step 3
Evaluate performance

Ultrafiltration/diafiltration

Although flux excursions provide initial information on device performance, it is essential to evaluate TFF in a realistic process: an ultrafiltration/diafiltration (UF/DF) study. Ultrafiltration confirms performance at higher virus titer, whereas diafiltration can provide further information on flux stability and fouling. Ultrafiltration evaluation is key here, with diafiltration providing additional information if time allows.

AAV yield evaluation

- AAV yield is a key driver for TFF processes, often dictating device choice.
- Yield evaluations can be performed in conjunction with UF/DF studies by measuring titer before and after, and performing a mass balance.

Impurity removal

- Although impurity removal is not typically a key driver for TFF with AAV, it may provide a reason to choose one device over another, especially before affinity chromatography.
- Impurity removal is readily evaluated with the UF/DF step by measuring impurity concentrations before and after, and by performing a mass balance.

Step 4
Usability comparison

CELL & GENE THERAPY INSIGHTS

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COMMENTARY

Don't play roulette with your clinical vector candidates: rethinking vector design for successful gene therapy

Alan Griffith

In today's fast-paced, high-stakes world of cell and gene therapy (CGT), where capital is fleeting and timelines are tight, success favors those who treat vector candidate selection not as an academic gamble, but as a scalar science. The past two decades for advanced therapy medicinal products (ATMPs) have laid bare a jarring duality: on one hand, highly investable and transformative emerging therapies, but on the other, tranches of developers chasing crowded indications with limited differentiation between actual product treatments, and many repeating the same mistake of rushed candidate selection.

Intensive pressure emerges as therapies targeting the same diseases race to trials, shifting focus to how quickly a therapy can move toward the finish line and disregarding red flags that foretell failure along the way. Investors target a potential therapy just after proof-of-concept (POC) of a single candidate with limited access to realistic indicators of success, so bets are placed early on, and inevitable hurdles are pushed past as quickly as possible. The result is a candidate that stumbles or falls in preclinical toxicology or full-scale GMP manufacturing due to issues that could have been predicted or prevented way upstream. The adage 'start with the end in mind' is often invoked to portray holistic thinking, but in practice represents a poorly executed aspiration. In truth, only a handful of developers truly have the depth of experience (e.g., millions of vector designs to draw upon) and the capabilities required to genuinely 'start with the end in mind'.

The solution lies not in speed, but rather in placing smarter, broader bets on candidate vectors. Betting everything on a single construct chosen too early and without sufficient rigor is like putting all your chips on a single number on the roulette wheel and hoping for the best. For those hoping to find success in CGT, we require more than speed and willingness to place high-stakes bets; we must be deliberate from the start. This sounds simple and sensical, but for developers without deep experience in vector design or empirical data, it is their key challenge.

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A SECTOR-WIDE CALL TO ARMS FOR IMPROVED VECTOROLOGY RIGOR AND READINESS

Due to the relatively low barrier of entry for vectors to transition from preclinical studies to candidate selection, developers can quickly test a gene of interest (GOI) in a standard vector and aim for efficacy-based POC rapidly. This single vector then becomes the focal point —the million-dollar bet is on. As a developer, you feel content, you feel like you are making real progress, your investors are happy, and a milestone has been reached. In reality, this bet is placed largely blindly. Numerous vector modifications are still required to address essential variables from efficacy to CMC scalability. Factors such as host cell decoherence, homologous recombination, and genome escape can ultimately prevent the program/product from meeting critical quality attributes (CQAs), specifications, and regulatory approval.

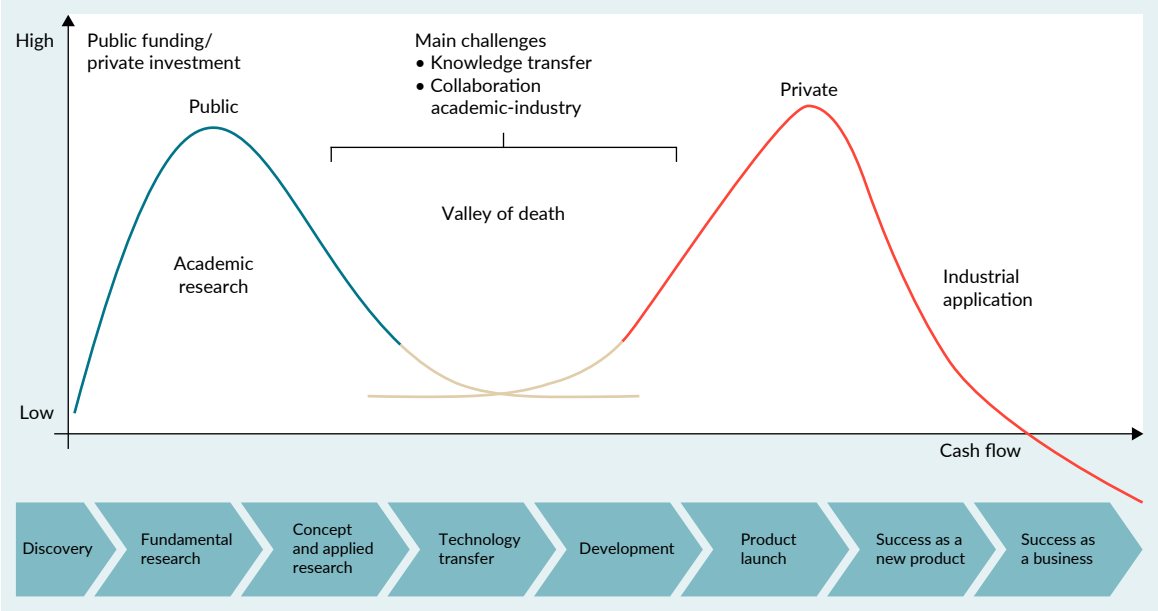
In many cases, the root cause is related to these variables in programs where vector design principles were not applied or addressed during preclinical stages, resulting in project pauses to redesign the vectors.

This redesign can take many forms, from simple modifications to total revamps. For example, often GC content must be reduced (silencing), repetitive sequences must be eradicated or reduced, and sometimes the entire vector backbone must be changed (beta-lactam switch to non-beta-lactam antibiotic markers). This can lead to significant changes to the candidate itself, but also major setbacks in both time and money, sometimes including going back to preclinical testing. In the world of drug development, this can mean the life and death of a multimillion-dollar program. This is now so pronounced that 90% of CGT clinical trials fail primarily due to lack of efficacy and toxicity.

The transition from discovery or POC to clinical application is often described

FIGURE 1

The typical flow of products through development and clinical translation highlighting the foreseeable 'valley of death', which many products succumb to.



as a leap over the infamous 'valley of death' (Figure 1). This critical juncture between research and first-in-human trials is where promising therapies too often fail, not necessarily due to flawed science, but due to insufficient rigor in early vector design, poor vector characterization, and manufacturability-related impurities. As more AAV therapies advance into the clinic, their complications are becoming clearer and increasingly well-characterized. One of the most concerning examples is thrombotic microangiopathy (TMA), a serious adverse event linked to high-dose systemic AAV.

There are two main bottlenecks (Figure 2) to bringing a gene therapy vector to late-stage clinical phases and subsequently market authorization based on the trends we have seen in the field over the last 10–15 years: vector design and CMC challenges (manufacturing and QC). Addressing these challenges in tandem and early in the CGT drug development pipeline is critical. By doing so, developers can avoid many of the pitfalls that have derailed otherwise promising therapies.

THE HIDDEN COST OF UNDER-OPTIMIZED VECTORS

What must change is clear: growing hesitancy now threatens the business viability of the CGT sector. The traditional playbook from small molecules and biologics—the race to generate clinical data on safety and efficacy, then partner or sell to big pharma—is proving unsustainable in the CGT context, there is still too much cost risk and the breakneck speed has led

FIGURE 2

Clinical 'holds' or, in extreme cases, ATMP failures can be attributed to two main bottlenecks, design of vector and manufacturing (CMC) challenges, which are intrinsically linked.

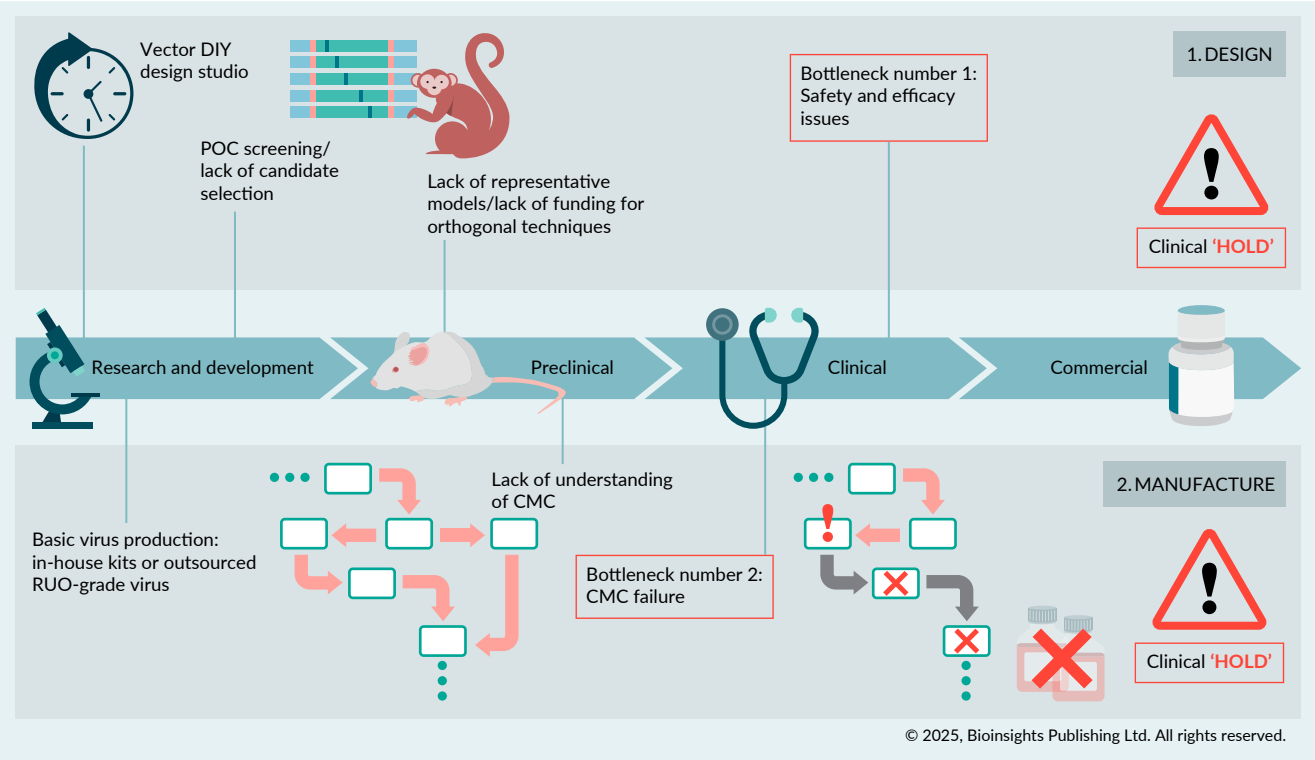
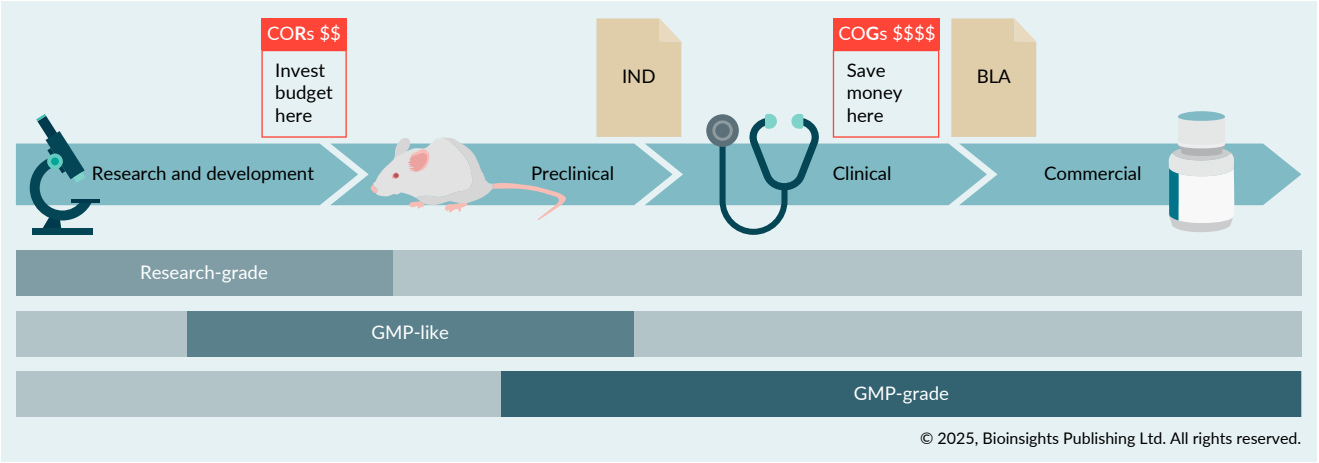


FIGURE 3

The development cost window goes from very modest to exponential beyond a few development stages (process development and scale up)—developers will never get this time window back, so they should utilise it with expanded vector design iterations using GVP.



to issues manifesting, and now the perception is worse than ever across the sector [5] meaning the weakest year on record for investment and only one initial public offering (IPO) this year.

Too often, CGT programs begin with a singular vector candidate, selected hastily and advanced with minimal optimization or comparative analysis. While this 'single bullet' approach may feel efficient, it overlooks critical variables that determine efficacy, safety, scalability, and ultimately, regulatory approval. The consequence? A candidate that stumbles—or fails outright—during preclinical toxicology or GMP manufacturing, with these predictable issues incurring rapidly escalating remediation costs.

This hasty mindset remains prevalent in early-stage biotech and academic institutions, where there is a stubborn belief that once an asset reaches a key inflection point in development, it can simply be offloaded in a co-development and/or co-commercialization partnership—essentially assuming that manufacturability of the asset will become someone else's problem.

The reality is different. For now, big pharma and venture capitalists/investors are largely sitting on the sidelines until the CGT industry can demonstrate it can deliver commercially viable products. Developers must strike a balance between speed to clinic and long-term product viability. Without it, CGTs risk being remembered as a fantastic science project that never lived up to its potential.

This is the 'clinical roulette' that CGT developers can no longer afford to play. 32 ATMPs have received EMA marketing authorisations, and 7 out of 32 (~22%) have since been *withdrawn* from the market, most citing commercial viability [10]. This is unacceptable; we must do better earlier in development.

Yes, it requires greater upfront investment in vector construct designs, testing, and screening—but those early costs reduce the risk of far greater expenses and failures downstream (Figure 3). It is unlikely that any developer will go back a step at this stage, and it is a shame that the stage where costs are lower (cloning and screening are cheap in comparison—ask anyone who has been to a clinic with an AAV, for example).

TABLE 1

Gene Delivery systems and considerations.

Viral vector composition	Conventional options for delivery system	Description
Vector system	Viral	Ease of introduction, but labor- and cost-intensive production
	Non-viral	Cost-effective but more difficult to introduce to cells
Gene expression	Stable integration	Life-long expression, but could result in insertional mutagenesis
	Transient	Low risk of host genome disruption, but expression is short-term
Backbone type	Standard	Cost-effective but may have safety concerns
	Miniaturized	Minimal bacterial components, but more costly
Antibiotic selection marker	Ampicillin	Standard for use in labs, but must be removed for clinical use
	Kanamycin	Safe alternative, but low risk of horizontal gene transfer
	None	Safest alternative, but requires another selection mechanism
Components	Promoter	Ubiquitous, well-studied, tissue-specific, or inducible, offer higher safety
	Linker	2A provides higher expression, but IRES is less likely to disrupt function
	Enhancer	Can increase expression, but may not have regulatory compliance

INTRODUCING GOOD VECTOR PRACTICE (GVP)

Recognizing the critical importance of vectors in research and medicine, and the high prevalence of errors in laboratory-made plasmids [11], here we recommend establishing a framework for good vector practice (GVP): a systematic approach to vector quality and robustness inspired by a similar school of regulatory compliance (GxP). GVP encompasses every phase of a vector's lifecycle, from initial design and cloning through in-process and product QC, all the way through to long-term vector banking. We believe that the adoption of GVP by the research community can improve the quality of vectors in laboratories worldwide.

From inception, GVP was conceived as analogous to GMP (where vectors are ultimately destined for). This is a new paradigm philosophy and framework for vector quality, with a simple but critical goal: to design, validate, and deliver the right vector for the right application consistently, at every stage of research and development. In practice, these key considerations include choice of vector system, backbone type, antibiotic selection marker, regulatory elements, and codon optimization for enhanced expression, stability, and cloning efficiency. Table 1 outlines some considerations for each of these points for expression vectors, providing a practical primer for GVP principles.

Next, we propose the systematic application of GVP principles across these key design considerations to create a rational, evidence-based decision-making process for your individual vectors. Below is a set of guiding principles we recommend incorporating into early design. While not exhaustive, they highlight common pitfalls and best practices that can substantially improve downstream performance, safety, and manufacturability.

GVP principles for rational vector design:

- Antibiotic selection: avoid antibiotic markers where possible. If required, replace β-lactam-related ampicillin with lower-risk alternatives such as kanamycin
- Regulatory elements: replace oncogenic WPREwt with safer alternatives Opre/WPREmut6/WPPRE3, which will either remove or hinder the X protein open reading frame
- Sequence provenance: ensure origin of fundamental sequences used by all transfer and gene of interest (GOI) plasmids are documented (origin story is needed for IND and GMO submissions)—choose your elements carefully
- Sequence integrity:
  - Remove cryptic slice sites and restriction sites not for downstream analytical or application use
  - Reduce GC (CpG) content where possible (or maintain sequence homeostasis)
  - Eliminate extraneous, non-functional sequences (e.g., f1 ori)
  - Avoid homopolymeric repeats (AAAA, CCCC, TTTT)
- Codon optimization: do not perform codon optimization unless the wild-type gene has a CAI of >0.75
- Vector size: aim to keep plasmids below 11 kb to improve fermentation and manufacturing yield
- Sequence source: perform *de novo* synthesis over legacy vectors of unknown provenance (do not accept gifts from strangers/collaborators)—legacy constructs have been shown to be poorly annotated/sequence errors and shared almost too freely between groups who use as a chassis for their respective indications—a recent study details the Prevalence of errors in lab-made plasmids across the world in CGT [11]
- LTR/ITR integrity: ensure ITR/LTR integrity from the outset, as these palindromic sequences are predisposed to truncations and deletions
- Element spacing: maintain interstitial space between functional elements (rather than juxtapositioned or too proximal; e.g., insert bases between polyA and 3' ITR)—in other words, positional effects can make a huge difference in vector performance
- Payload-to-backbone ratio: ensure payload and backbone ratio is considered (e.g., some vectors will benefit from a ratio of 2:1 [payload: backbone]) to assist with reverse packaging in AAV, some will require more ratio optimization during process development/design of experiments studies)

While some design pitfalls are more common, even seasoned molecular biologists may overlook subtle but critical nuances across these parameters [11]. The ability to identify and proactively address such issues is highly dependent on both breadth of experience and access to empirical data. Therefore, a tenet of GVP urges utilization of available resources early—whether through trusted knowledge hubs, direct expert consultation, or speaking with developers who have been down this road before.

In addition to choices in design, a common challenge arises when sourcing both components and backbones. For instance, multiple versions of the CMV promoter with various lengths and specific sequences exist

in circulation, leaving developers uncertain about which version to select or how to document accurately for a regulatory submission. Many vectors reach POC utilizing research-grade materials, including legacy plasmids sourced from colleagues, institutes, and pioneering founders. While these hand-me-down constructs may be convenient, many of these plasmids were never optimized for key performance attributes, such as empty/full packaging ratios. This heterogeneity in rAAV vectors can elicit both innate and adaptive immune responses, limiting efficacy and potentially leading to adverse effects. Additionally, these vectors may harbor mutations from rounds of amplification.

A key aspect of designing GVP-compliant vectors intended for clinical use is the enhanced requirement for safety, efficacy, and manufacturability. When designing a clinical-grade vector, safety is paramount, and certain components commonly used in research vectors may need to be substituted to meet regulatory requirements on safety in human use. For instance, the frequently used ampicillin resistance marker on plasmid backbones should be replaced with kanamycin resistance or a miniaturized antibiotic-free plasmid to avoid allergenic potential. Similarly, the regulatory element WPRE, often used to enhance expression, may need to be substituted with safer varieties (e.g., WPREmut6 or WPRE3) due to its potential oncogenic risk [1,2].

GVP TO SAFEGUARD VECTOR CMC FOR CLINICAL TRIALS & BEYOND

To achieve optimal clinical performance, vectors should be more comprehensively optimized for regulatory compliance at the early stage, with careful selection of coding sequences, promoters, and regulatory elements. Furthermore, accounting for CMC/manufacturability in clinical-grade vectors enhances yield, long-term sequence stability, and the percentage of supercoiled plasmid without the lost time and money of performing these optimizations after preclinical stages. When initiating a plasmid cloning project, it is essential to verify the fidelity of DNA templates used as starting materials, especially if they originate from sources that do not routinely perform stringent QC, such as laboratory peers and collaborators. Assays such as diagnostic restriction digestion, sequencing, and other adequate QC tests should be performed as needed to ensure that the starting DNA materials are consistent with their theoretical sequence and structure. Additionally, in-process QC should be considered for all steps involving DNA synthesis (e.g., reverse transcription, PCR, and Gibson assembly) to mitigate the risk of mutations and should pass QC before proceeding. Product QC should also be performed for assets that are vital for downstream applications. Packaging plasmids and sequences on viral vectors that function in packaging but not downstream biological applications, which tend to get overlooked, should be confirmed to ensure proper viral packaging. This is especially the case for AAV inverted terminal repeats (ITRs), which tend to mutate during fermentation, and developers cannot rely on ITRs self-repairing during virus production or *in vivo* [4]. These assays are simple and cost-effective but are often overlooked in the interest of achieving POC first and foremost. The feedback from the agencies over the last decade has been that more characterization is needed, especially at the protein level of these viral vectors; for example, the MHRA is bringing out new guidance this year covering vector/capsid protein characterization specifically.

While regular and thorough QC is imperative to ensure fidelity of materials involved in CGT development, maintenance of these standards and traceability requires a free-standing vector banking and management system. Plasmids in any

given laboratory, whether sourced or self-built, should have unique IDs, descriptive names, and complete records including creators, maps, sequences, cloning methods and steps, and QC dates and results. Proper archiving of this information is critical for troubleshooting any issues that may arise in downstream applications. For plasmids retrieved from frozen stocks that have not been used for a long time, it is important to re-validate them to ensure consistency with past records and update relevant information.

As a key aspect of the academic ecosystem to maintain vector quality, we suggest that journals encourage authors to utilize GVP principles when publishing. This would significantly raise vector integrity in research and medicine.

REAL-WORLD EXAMPLES & HOW TO BUDGET FOR INCREASED VECTOR CANDIDATES

We recognize that what is presented here demands more of the developer. However, recent headlines provide a strong motivator: poorly performing ATMPs repeatedly face clinical holds or outright failures due to preventable shortcomings [6–9]. Common themes include suboptimal vector designs, weak preclinical dosing strategies that relied on less potent vectors, and poor process integration. As summarized in Table 2, the consequences of overlooking vector quality and integration early can be severe.

Employing GVP principles in design, production, QC, and data management allows for the creation of a strong candidate vector that can be a safer bet in CGT development. It should not be seen as a penance, but rather an opportunity to deliberately optimize key attributes critical to the clinical and commercial success of each vector candidate, such as:

- Transgene expression levels in target tissues
- Tissue specificity and minimization of off-target effects (liver detargeting)
- Immunogenicity and toxicity profiles
- Manufacturability under larger-scale GMP conditions/process

TABLE 2

Current CGT trails on hold or experiencing issues related to CMC or vector unresolved issues.

Company	Product/indication	Hold type	Timing	CMC issue description
Ultragenix	UX111 (AAV MPS-IIIA)	CRL	July 2025	Facility/process observations
Rocket Pharma	RP-L201 Kresladi (LAD-I)	CRL	June 2024	Requested additional CMC/facility data
Solid Biosciences	SGT-001 (DMD)	Clinical 'hold' → lift	2020	Manufacturing process, viral load control
4D Molecular	4D-310 (Fabry disease)	Clinical 'hold'	2023	Safety events plus associated manufacturing follow-up
Voyager Therapeutics	VY-HTT01 (Huntington's)	IND 'hold'	2020	Pre-initiation CMC deficiences
Sarepta	SRP-9001-103 (DMD)	Clinical 'hold'	2021	Plasmid fragment trace due to CMC issues
Outlook Therapeutics	ONS-5010 (Wet AMD)	CRL	August 2023	CMC, pre-approval inspection failures

- Stability and robustness across delivery modalities

Expanding your candidate pool may mean a higher upfront price per clinical candidate (PPCC). However, this metric becomes a compelling communication tool for internal stakeholders and investors when framed correctly. Investing more at this stage protects against costly failures later. The PPCC mindset reframes vector selection not as a cost center, but as a value creation opportunity: minimizing program attrition, avoiding redesigns, and increasing the chance of IND success. It goes back to Figure 3—budget now, and let the payers understand you are doing it for a reason. Use PPCC as a budgetary line item to seek approval. It is apparent that too often, developers are bringing vector designs from early research and preclinical studies without sufficient vector design rationale and empirical validation. It is no secret that investors and VCs will demand as fast a route as possible to final candidate selection so they can engage their CDMOs of choice or start process development activities in-house, depending on their mode of manufacturing. This approach of speed over scientific rigor has produced a CGT field blemished by the gravestones of once-promising therapies that fell victim to rushed and ineffective solutions.

The most pressing issue in gene therapy today is the conflict between scalability and affordability. High manufacturing cost of goods (COGs), dose-related safety concerns, and limited durability in some tissues and market reimbursement challenges are all cantilever issues, leaving drug developers with more problems than solutions. Scaling up the manufacturing of ATMPs is a multifaceted challenge involving technical, regulatory, and financial aspects. The most critical scale-up concern for ATMPs is demonstrating product platformization and comparability after manufacturing process changes; by understanding this early, you gain a crucial advantage in a very competitive field.

CONCLUDING SENTIMENT

This article argues for a fundamental shift in how CGT programs approach vector design. Too often, developers rush the transition from laboratory to clinic—pushed by investor pressure and unrealistic boardroom expectations—by advancing the first vector that shows efficacy *in vitro*. The past decade has shown where that road leads: stalled programs, costly redesigns, and avoidable clinical failures.

The communication gap between molecular DIY cloners and the key decision makers may never be bridged; therefore, adherence to GVP and moving away from conventional development (by creating more vector constructs early in the development lifecycle to hedge your bets) at least gives developers a higher success rate overall. It also arms developers with a counterargument to investors and gives the people who hold the purse strings a reason why it is better to have less haste during candidate selection and set the expectations for inflection points differently on their respective clinical development Gantt charts.

Ultimately, it highlights the risks of inadequate vector development, which can lead to therapy failures, and advocates for a systematic, evidence-based approach to improve clinical outcomes.

"For far too long, drug developers have been playing a guessing game when navigating the FDA," said FDA Commissioner Marty Makary. "Drug developers and capital markets alike want predictability. So today we're one step closer to delivering it to them, with an ultimate goal of bringing cures and meaningful treatments to patients faster." [12].

In a field driven by urgency and investor expectations, too many developers spin the wheel with a single, unoptimized vector and

hope for the best. It is time to rethink this gamble. Diversify early, design deliberately, and make vector optimization a strategic pillar, not an afterthought. This shift in mindset will allow both developers and investors to avoid tossing their chips and pinning their hopes on a single space, and instead take well-informed, forward-thinking steps from the beginning, paving a smoother path to the clinic.

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CELL & GENE THERAPY INSIGHTS

INNOVATION INSIGHTS

How AI and precision medicine could transform women's health

VIEWPOINT

"AI-driven models can detect subtle variations in how women respond to certain medications, or how autoimmune diseases present differently across genders."

A two-week travel diary uncovering the market potential in addressing the gender gap, by **Helena Strigård**, Founder, Ventures Accelerated.

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Women's health, and the massive market potential that ought to lie in addressing it, has been pre-occupying my mind for over the past year. Although women's health is often narrowly defined as conditions exclusive to women such as fertility issues, gynecological cancers, or menopausal symptoms, this definition misses a broader and more urgent reality: how many diseases affecting both men and women manifest differently in women. How have these discrepancies been unrecognized, under-researched, and under-invested for so long, and what it would take to change that?

Take heart disease, for example. Long considered a 'man's disease', it remains the leading cause of death for women. Yet, many women experience subtler, non-typical symptoms—such as fatigue, nausea, or jaw pain—that deviate from the textbook chest pain presentation more common in men. These differences have historically led to misdiagnosis or undertreatment.

Similarly, in conditions like diabetes, studies have shown that women may have a higher risk of heart complications than men at similar glucose levels, yet treatment strategies have often failed to reflect these distinctions or other hormonal effects.

However, we are starting to see signs of a major game changer coming to address this issue: AI—the two letter abbreviation we have rapidly seen entering field after field of our economies. We are beginning to see how AI and precision medicine together, including technologies in cell and gene therapies, could revolutionize healthcare. By analyzing vast and diverse datasets, including genetic profiles, clinical histories, social determinants, and even wearable device data, AI could identify patterns that reveal how diseases affect women differently than men. For instance, AI-driven models can detect subtle variations in how women respond to certain medications, or how autoimmune diseases present differently across genders.

For 2 weeks, I had the opportunity to tour the Californian life science ecosystem together with a film team from Phosworks. During our 'Venture Road California', we drove from San Francisco to San Diego, interviewing frontrunners of life science innovation. Over the course of the tour, it became abundantly clear that the intersection of precision medicine, AI, and women's health presents a sweet spot for both Californian and Nordic ecosystems. These places share an inherent culture of encouraging innovators to roam free more generously than elsewhere, and a sense of the sector convergence that happens when experts from disparate fields such as IT and biotech squeeze together in overlapping networks. They also share a genuine value-driven appreciation of diversity in all its forms, including diverse thinking.

This seems to have paid off, with Sweden being the number one most innovative country in the EU and ninth most important investor to the USA globally, and California recently hitting the mark as the world's fourth largest economy. Talking to numerous Bay area innovators, they share my excitement for what can come out of the marriage between precision medicine and AI. We dive into how this all relates to the 'different in women-aspect' of women's health.

One of them is Dr Karin Rosén, who moved from Sweden to California pursuing a career as physician and researcher whilst embracing roles in the vibrant biotech community in both California and the Nordics. In her capacity as a Board Member of the Swedish company, Diamyd Medical, we sat down and talked about the market opportunity that lies ahead and the enormous market that remains to be unveiled. "Boosting data availability, care delivery, investment and treatment for selected health conditions that impacts women including cardiovascular disease could create nearly US$400 billion in annual economic improvement by 2040," says Rosén with reference to data from World Economic Forum [1].

For Diamyd Medical, developing a precision medicine to prevent destruction of insulin producing cells, this market is highly relevant as diabetes Type 1 is one of the medical conditions that manifests differently in men and women.

> "Diamyd® targets patients with autoimmune diabetes (Type 1), who have a specific genetic HLA DR3-DQ2 haplotype. Some 10–20% of the more than 537 million living with diabetes suffer from autoimmune diabetes, and Diamyd is now being investigated in a global clinical Phase 3 trial and has earned Orphan designation and Fast track designation from the US FDA."

She continues to explain how women with Type 1 diabetes have close to ten times higher risk of cardiovascular disease (stroke, heart attack or CV death) and how AI is a natural tool for them in their precision medicine approach to the disease:

> "AI algorithms can identify individuals at higher risk of developing Type 1 diabetes, allowing for more timely interventions to slow down the disease progression and thus prevent organ damage. US market research, including interviews with US payers, demonstrated a strong willingness to consider prescribing Diamyd for Type 1 diabetes and with a peak sales potential of up to US$2 billion."

Why is the untapped potential of addressing 'different in women' with a precision medicine approach not exploding as a research field and market? With AI developing rapidly, this should be dynamite. I decided to turn to someone who speaks the language of investors, one of the most disruptive thinkers of the Nordic financial landscape: Nina Rawal, Partner and Co-Head of Till Impact's Venture arm. She explained how to unlock capital into an AI-powered precision medicine approach to women´s health.

> "Addressing women's health is a crucial economic opportunity, not just a moral imperative. Decades of underinvestment and bias have created a trillion-dollar blind spot, which the World Economic Forum estimates could add at least US$1 trillion to the global economy annually by 2040. AI offers a unique chance to leap ahead, but seizing this opportunity requires a deliberate strategy. We must build early success cases to prove AI's potential and attract greater investor appetite. Together with Dorothy Chou from Google DeepMind, my colleague Bita Sehat and I just finalized a paper entitled: 'From margins to momentum: an AI-enabled transformation in women's health', summarizing this opportunity" [2].

Some of the enigma remains though, why are we not moving faster? And more importantly, what could an angel investor from Sweden possibly do to help accelerate ventures in this field? An idea begins to form...

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Helena Strigård holds a MSc in Molecular Biotechnology Engineering and a BA in Management. She is the Founder and CEO of Ventures Accelerated, a conglomerate of seasoned life science investors and executives working to enable companies and investors to access capital and target-markets. Prior to this, Strigård held leading positions in the industry, including as CEO and partner of Haeger & Carlsson Executive Search and Interim, CEO of Swedish-based CDMO NorthX Biologics, and Director General of SwedenBIO, the Swedish life science industry organization. In her current undertaking, Strigård is building on her previous experience in the public sector, including as Head of Section at the Finance Ministry of Sweden between 2012–2017, as well as her roles in industry, to support life science companies from all over the Nordics and beyond on their international growth journey.

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CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Turbocharging plasmid DNA production: a case study on optimization through novel technologies

Xiangming Gu, Lucas Smith, James Rankin, Donald Belcher, Frank Agbogbo, and David Dismuke

The growing demand for high-quality plasmid DNA in gene therapy and DNA vaccines necessitates improvements in manufacturing processes. This study explored optimizing tangential flow filtration (TFF), following cell lysis and depth filtration, by evaluating filter types, shear rates, and automation features. The implementation of a fully automated TFF system paired with the ideal membrane format reduced processing time by 300% and enhanced RNA removal while preserving supercoiled pDNA. Optimal shear rates further improved efficiency. Additionally, scale-up using single-use TFF for pre-chromatography and final formulation steps was assessed. The results highlighted the value of automation and real-time monitoring in maximizing recovery and maintaining product quality, offering a more efficient, scalable pDNA production platform.

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CURRENT cGMP PATHWAY PLATFORM

The current cGMP pathway plasmid DNA (pDNA) production workflow, especially the midstream and downstream steps, includes the following unit operations: alkaline lysis, clarification, and tangential flow filtration (TFF) I. The TFF I acts as a pre-chromatography step for volume reduction and conditioning. Next, a two-stage chromatography process is used to achieve high purity and effective impurity removal. The final formulation of TFF allows buffer exchange and plasmid concentration. The process ends with sterile filtration and final filling. This method has reliably produced a high percentage of supercoiled plasmid, along with high levels of recovery in multiple runs. This shows the robustness of the downstream steps but also emphasizes the need to optimize and automate key steps like TFF to maintain performance during scale-up.

PROJECT SCOPE

A study was conducted to evaluate a fully automated system for both pre-chroma-

tography TFF I and post-chromatography TFF II steps during the plasmid purification process. The aim was to understand how automated TFF can impact pDNA recovery and product homogeneity across a workflow. Other objectives included comparing the performance of hollow fiber and flash sheet membranes in TFF I and testing the system in final formulation TFF II to assess flexibility, performance, and overall compatibility with the existing workflow.

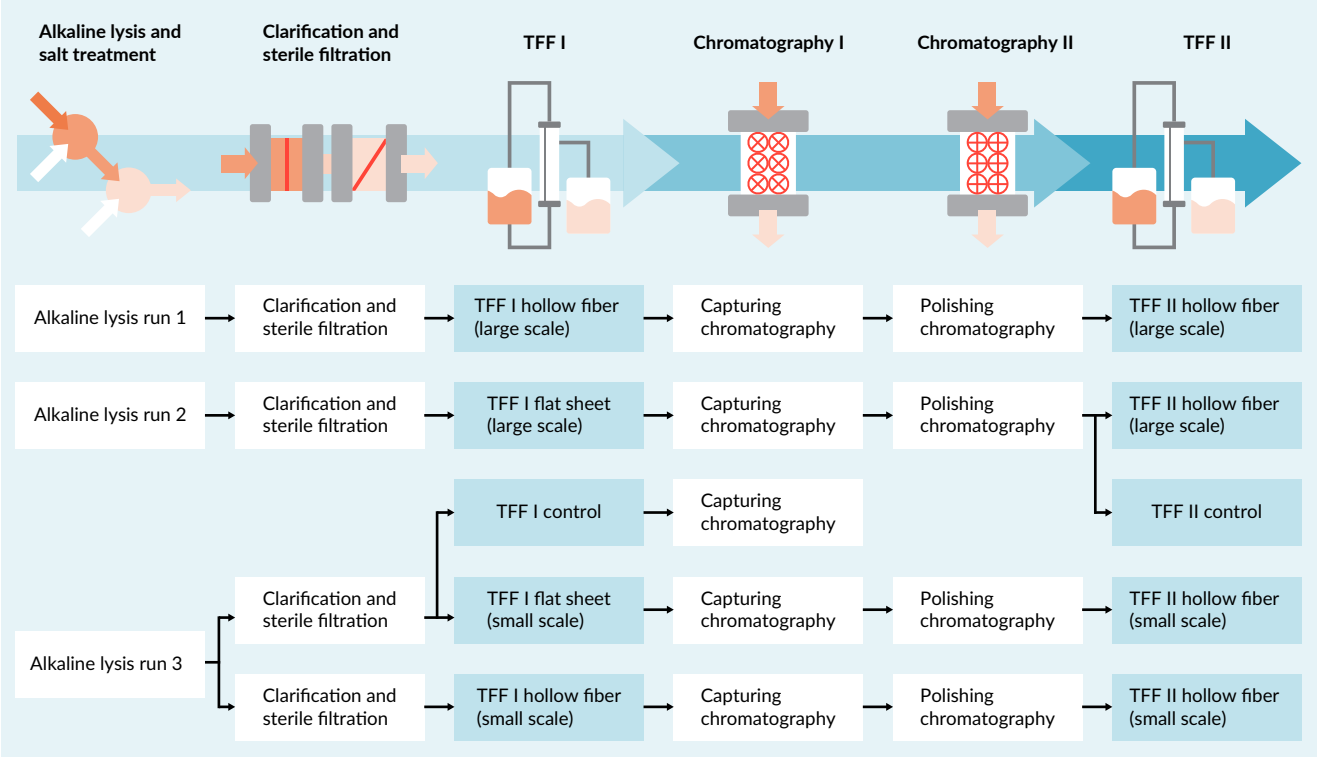
PROCESS AUTOMATION TO MINIMIZE DISCREPANCIES & PRODUCT LOSS

The **KrosFlo® RS 20 TFF system** by Repligen boasts several features that enable true end-to-end automation, reducing processing time and the need for manual intervention. Some of its features include a back pressure control valve harmonized in a control loop with a high accuracy feed pump, ensuring that the pressure and flow settings are optimized without operator intervention. This minimizes operator error and prevents issues such as transmembrane pressure (TMP) overshooting during the initialization phase of TFF, which could otherwise lead to inconsistent results. The system also uses numerous pneumatic valves enabling the system to automate leak testing, flushing, and sanitization steps, as well as diafiltration and concentration. UV is used to determine if there is pDNA loss from the permeate. The system can also measure pH and conductivity of the retentate to assesses if the final formulated material falls within the pre-defined release criteria.

Using a KrosFlo RS 20 TFF system also helps streamline data collection and calculation of parameters such as pressures, flow rate, and volume. The system can auto-populate relevant parameters based on the filter type selected, thereby reducing setup time

FIGURE 1

Study design to evaluate performance of a fully automated TFF I and II system.



and operator error. Additionally, the system can auto-track the retentate weight before and after diafiltration, which is particularly valuable if there are inaccuracies in initial volume estimates, ensuring accurate diafiltration without manual recalculations.

STUDY DESIGN

As seen in Figure 1, the performance of TFF I and II is evaluated through a fully automated system. A total of three alkaline lysis runs were conducted; two large-scale runs were performed in duplicate and served as the primary evaluation for full-scale performance of the system. The third batch was divided in half and processed separately for small-scale assessment, to assess the flexibility of the system with a reduced volume. Between TFF steps, a capture and polishing step was performed to ensure consistency in intermediate processing. As a benchmark, a control run was carried out using a semi-automated TTF system under equivalent conditions.

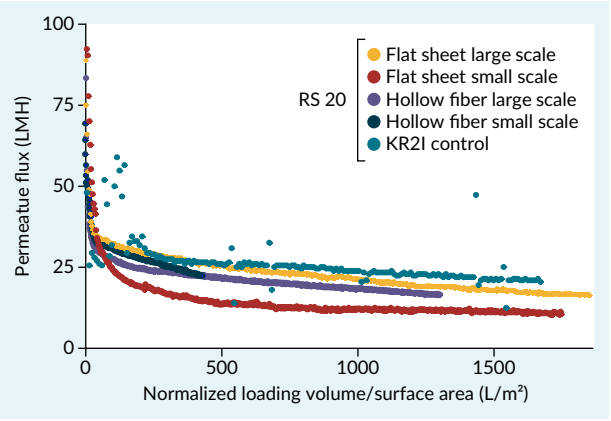
TFF I (PRE-CHROMATOGRAPHY): pDNA CONCENTRATION AND PREPARATION FOR CHROMATOGRAPHY I

First, flux behavior during the TFF I step was assessed. Consistent flux decay was observed during small- and large-scale runs using a hollow fiber membrane, indicating good scalability. Whereas for flat sheet cassettes, differing performances were observed for both small- and large-scale runs, both of which can be seen in Figure 2. This is likely due to the relatively large size of the pDNA, which may impact membrane interaction and retention behavior. Despite these performance differences, these results show that both hollow fiber and flat sheet membranes can be utilized using this fully automated system.

The next study analyzed pressure profiles to assess the operational consistency

FIGURE 2

Flux decay profiles using hollow fiber and flat sheet membranes during TFF I.



of hollow fiber and flat sheet membranes. **Spectrum® hollow fiber membranes** (Repligen) were operated under constant TMP control, showing stable TMP across both small-scale (60 L) and large-scale (120 L) runs, demonstrating good scalability and predictable performance (Figure 3A and 3B).

TangenX® flat sheet membranes, however, were operated under constant retentate pressure, reflecting a different operational approach that may impact shear conditions and membrane interactions, especially with large size of pDNA (>10 kbp) (Figure 3C and 3D). Collectively, these results indicate that hollow fiber membrane is preferred for TFF I.

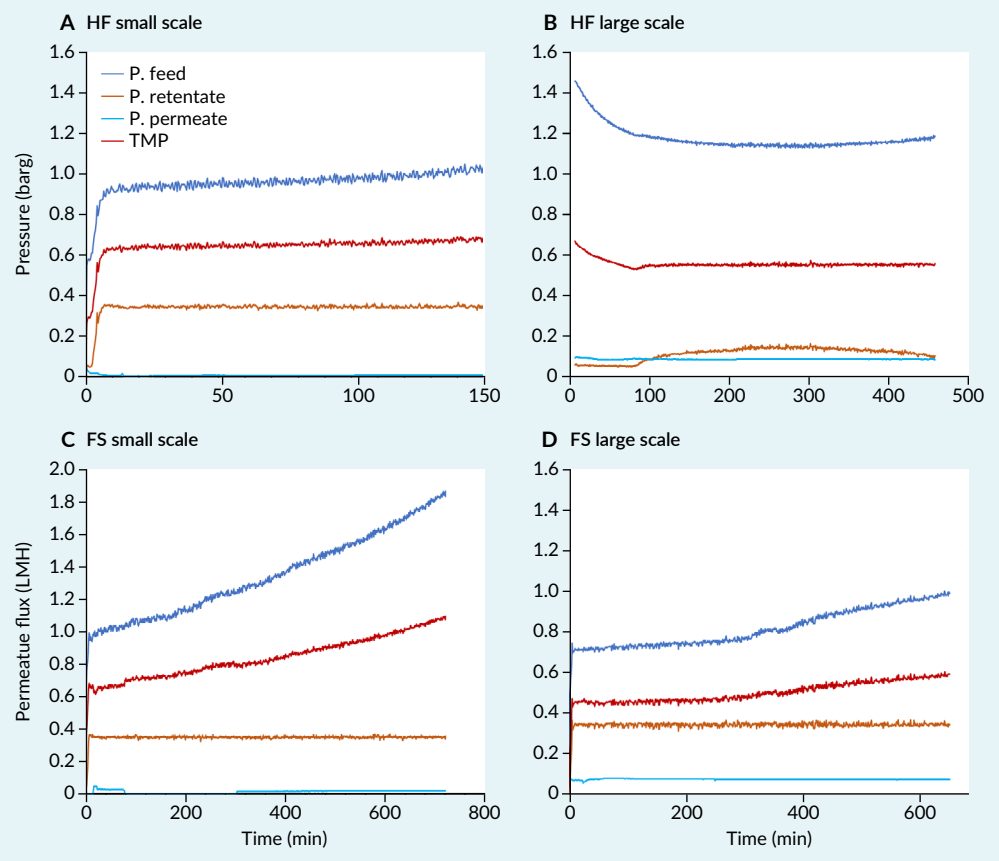
CHROMATOGRAPHY I (pDNA CAPTURE)

The next step was to assess pDNA recovery during chromatography I, following TFF I. pDNA recovery was performed using a single-use chromatography system designed for a cGMP environment.

Consistent pDNA recovery was observed with both hollow fiber and flat sheet membrane types across small- and large-scale runs (Figure 4). Interestingly, samples prepared with hollow fiber membrane showed

FIGURE 3

Pressure profiles assessing performance of hollow fiber and flat sheet membranes during TFF I.



greater RNA removal compared to flat sheet membranes. This suggests that there may be meaningful differences in impurity profiles based on the type of TFF membrane used. It was noted that more work is needed to optimize operation with flat sheet membranes. Furthermore, tests confirmed a strong alignment between small- and large-scale runs, demonstrating the reliability of this platform in reducing host-cell impurities following TFF I.

CHROMATOGRAPHY II (pDNA POLISHING)

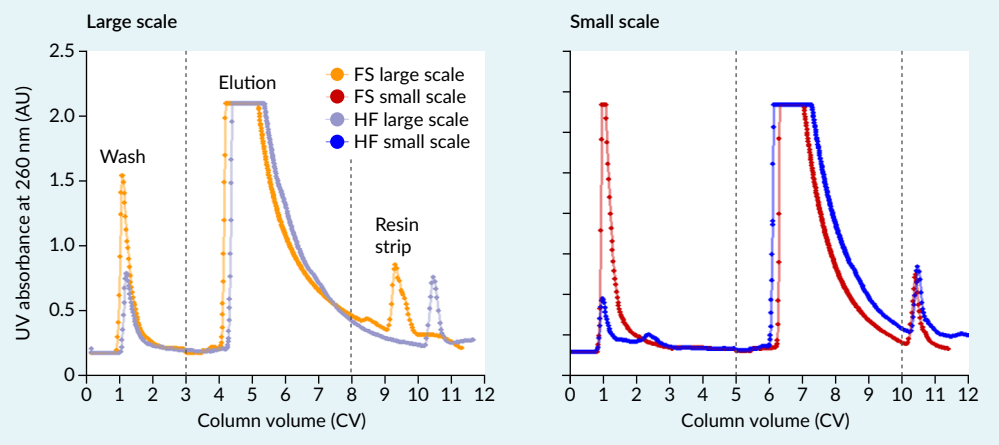
The chromatography II step was next evaluated. This step focused on the final purification and polishing of the pDNA product. The primary objective was to isolate high-purity supercoiled pDNA from a mixed population that also included open circular and linear DNA forms. The system was able to recover more than 90% of supercoiled pDNA from other isoforms (Figure 5), demonstrating effective separation and meeting the quality criteria. This shows that the KrosFlo RS 20 TFF system consistently produces high-purity supercoiled pDNA, validating its suitability for integration into the workflow.

TFF II (POST-CHROMATOGRAPHY)

The key challenge of the TFF II-final formulation step is the diafiltration and concentration of pDNA without compromising the integrity of the supercoiled plasmid. Since the current buffer used in the plasmid solution is

FIGURE 4

pDNA recovery during chromatography I with Spectrum hollow fiber and TangenX flat sheet membranes.

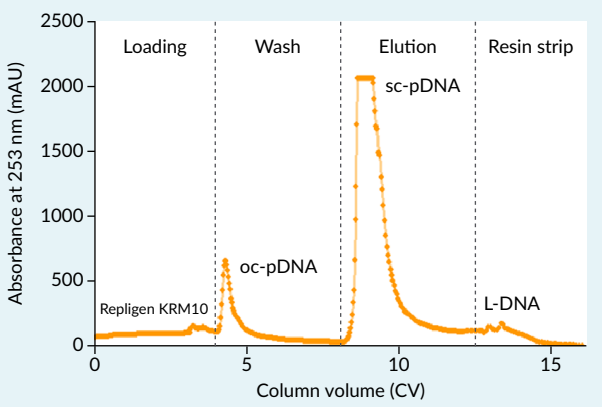


incompatible with cell culture, diafiltration and concentration are necessary to prepare the plasmid for recombinant AAV applications. The objective of this study was to evaluate the effectiveness of TFF II to perform buffer exchange and concentration without damaging the plasmid structure.

The results showed that high plasmid recovery was achieved (>90%) after the TFF II process, with minimal formation of open circular plasmid. This indicates that the integrity of the supercoiled plasmid was preserved. TFF II process demonstrated consistent pressure over time during diafiltration (Figure 6A). Furthermore, there was no significant flux decay at constant TMP, showing stable and reliable system performance (Figure 6B). A drastically higher flux was observed during TFF compared to a semi-automated TFF system (Figure 6C). This translates to a reduction in processing time, which is important for improving efficiency. TFF II proved to be a reliable and scalable solution for final formulation, delivering high plasmid recovery with minimal degradation compared to semi-automated chromatography systems. Flux and processing time were also greatly reduced compared to semi-automated systems.

FIGURE 5

Recovery of supercoiled pDNA during chromatography II.



Consistent recovery from RS20 system

CONCENTRATION OVER TIME (CTech™ SoloVPE® DATA SUMMARY)

Featuring patented variable pathlength technology (VPT), the **CTech SoloVPE** (Repligen) was used to measure plasmid concentration. Final plasmid concentration was measured to be 1 mg/mL, which aligned with internal release testing criteria. For the permeate concentration, a

FIGURE 6

(A) Pressure profile and (B) flux decay profile during TFF II and for comparison, (C) pressure and flux decay profiles achieved using a semi-automated system.

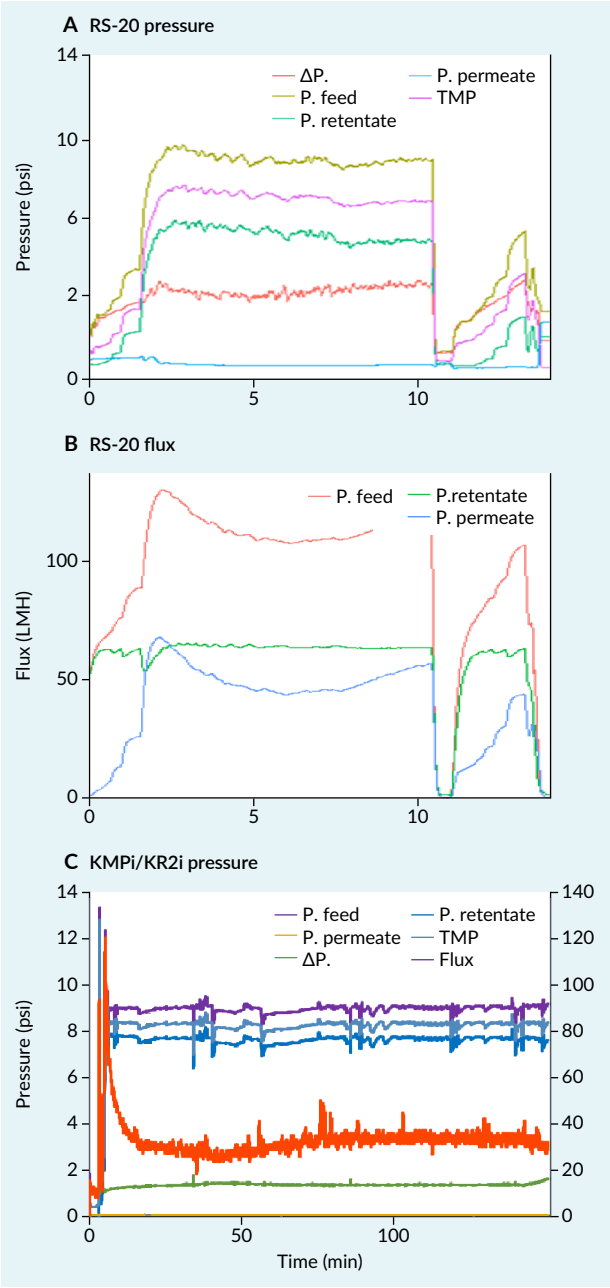
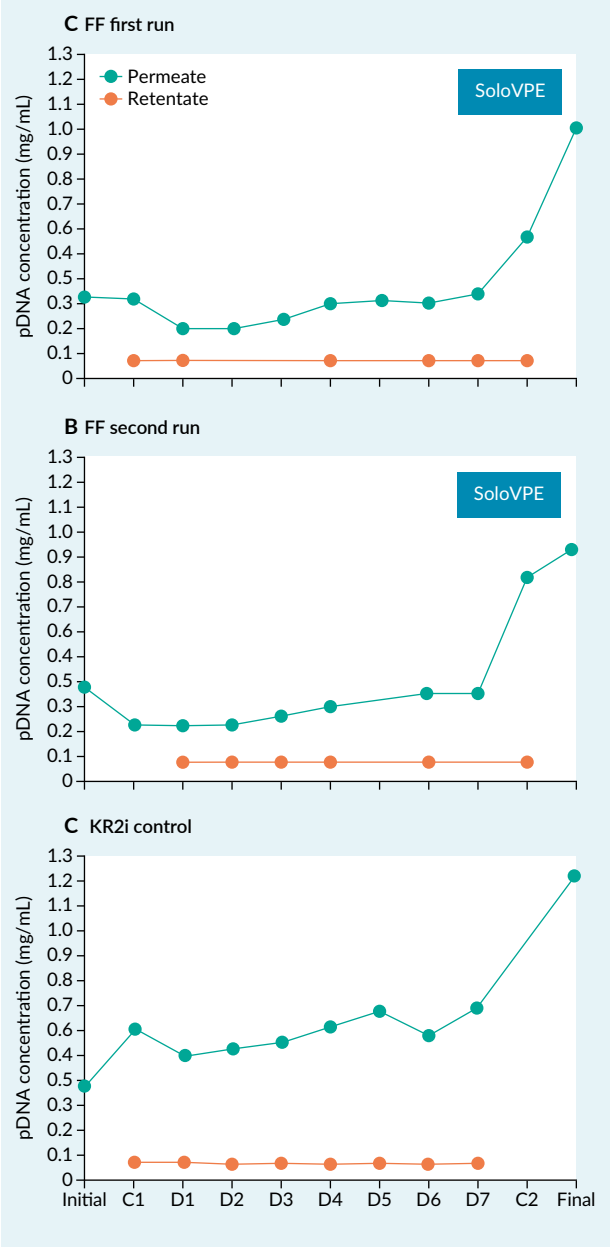


FIGURE 7

Measurement of plasmid concentration over time using slope spectrometry.



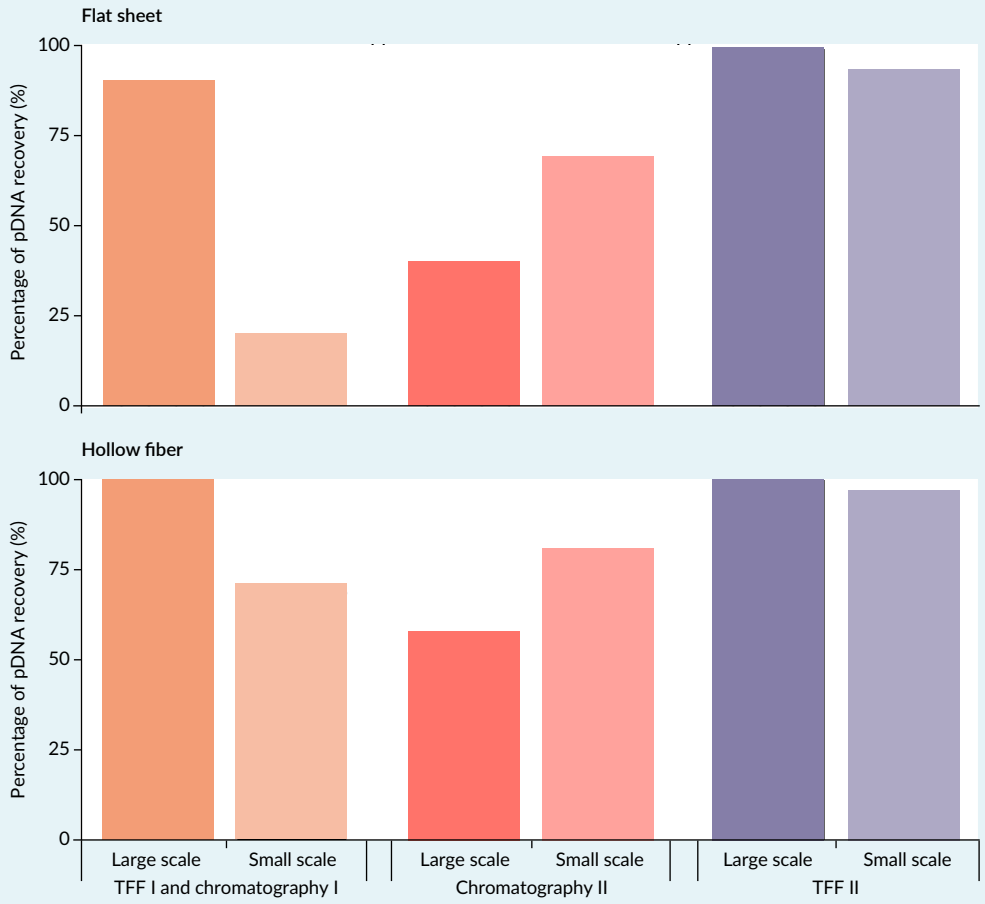
value below 0.01 Abs/mm was recorded, which indicated no detectable pDNA concentration at the permeate (Figure 7). This confirmed that there was no negligible loss from the permeate, demonstrating the effectiveness of the filtration process.

YIELD & RECOVERY

To understand the pDNA processing efficiency throughout the workflow, an evaluation of the recovery and yield over the major process steps, comparing hollow fiber and flat sheet membranes, was conducted. The results showed that hollow fiber membrane consistently

FIGURE 8

pDNA recovery during TFF I, Chromatography I and II, and TFF II.



Identifier	Process step	Absolute yield (mg)		pDNA recovery* (%)		Supercoil degradation (%)	
		Hollow fiber	Flat sheet	Hollow fiber	Flat sheet	Hollow fiber	Flat sheet
Large scale	Mainstream TFF	~7500†	~7500†	99	89	N/A	N/A
	Chromatography I	7400	6659	99	89	<2	<2
	Chromatography II	4227	2580	57	39	N/A	N/A
	Final formulation TFF	1750	1830	99‡	98‡	<1	<1
Small scale	Mainstream TFF	~3750†	~3750†	70	19	N/A	N/A
	Chromatography I	2632	703	70	19	<2	<2
	Chromatography II	2106	481	80	68	N/A	N/A
	Final formulation TFF	310	385	96‡	92‡	<1	7400

*The pDNA Recovery is defined as the percentage of the mass of pDNA for each individual step. †Calculated value based on historical specific yield (1.5 mg/g). ‡Approximate 1760 and 1860 g of pDNA yielded from the large-scale hollow fiber and flat sheet midstream TFF, respectively, were used for large scale final formulation TFF. Approximate 323 and 385 g of pDNA yielded from the small-scale hollow fiber and flat sheet midstream TFF were used for small scale final formulation TFF.

demonstrated higher step recovery across each unit operation. During the final formulation step, high plasmid recovery was observed during both small- and large-scale runs (Figure 8). This highlights the flexibility and scalability of the platform

TABLE 1

Measurement of plasmid purity at final fill stage using hollow fiber and flat sheet membranes.

Sample description	Assay	Criteria	RS20 HF	Result RS20 FS	KR2i control
Final filled plasmid	pDNA recovering post-TFF	Report result	>90%	>90%	>90%
	SC degradation post-TFF	Report result		<1%	
	Restriction digest (AsiSI, HindIII, AhdI, NcoI)	Banding matches expected pattern	All matched	All matched	All matched
	Appearance	Clear and colorless	TRUE	TRUE	TRUE
	pH	Report result	7.52 ± 0.07	7.27 ± 0.4	7.86 ± 0.01
	Residual	Report result	0.42%	0.66%	0.53%
	Residual RNA (SYBR Gold Gel)	Report result	<5% (below detection limit)	<5% (below detection limit)	<5% (below detection limit)
	Nanopore sequencing	Aligns with expected sequence	>99.8%	>99.8%	>99.8%
	Non-USP sterility	Pass	Passed	Passed	Passed

FS: flat sheet membrane. HF: hollow fiber filter.

using hollow fiber membranes. The recovery profile confirms the system's efficiency and reliability for both small- and large-scale pDNA production, therefore validating its integration into the workflow.

COMPREHENSIVE CHARACTERIZATION OF FINAL FILL SAMPLES

An evaluation of plasmid purity was conducted. Full panel analytical testing was performed on the final fill plasmid to ensure it was of high quality before proceeding to the next stage. Overall, the results, as seen in Table 1, showed negligible amounts of host-cell DNA and RNA present, confirming the successful removal of impurities during the purification process. Plasmid identity also remained intact, meaning no degradation or modification had taken place during the process, which is critical for downstream applications. Notably, a high supercoil percentage (>90%) was achieved, ensuring that pDNA remains biologically active and stable when undergoing these processes.

AUTOMATED PROCESS CONTROL MINIMIZED OPERATION AND ERROR, AND ENHANCED PROCESS EFFICIENCY

Implementing this automated system into the pDNA purification process has significantly improved processing time and consistency for both TFF I and TFF II steps (Figure 9). Automation of TFF I resulted in a 179% improvement in process efficiency by reducing the total processing time from 21 hours to 11.7 hours. For TFF II, process automation resulted in an almost 300% improvement in efficiency, cutting the total processing time from 17 hours to 5.7 hours. This reduction is crucial for scaling operations and meeting production demands in a more streamlined way.

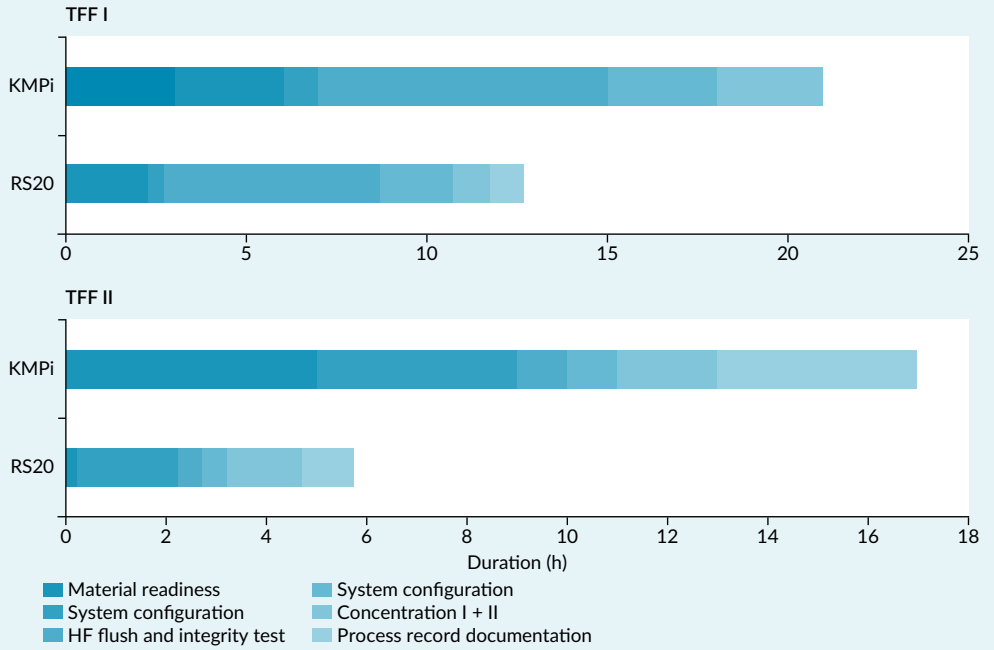
CONCLUSION

In conclusion, this work has outlined the successful implementation and optimization of the pDNA manufacturing process using the KrosFlo RS 20 TFF system.

Adherence to manufacturing criteria, product quality, safety, and regulatory compliance while boosting productivity were the forefront of the operations.

FIGURE 9

Depiction of improved process efficiency using a fully automated system for TFF I and II compared to a semi-automated system.



Q&A

Xiangming Gu (left), **Lucas Smith** (right)

Q What strategies have worked best for minimizing genomic DNA and RNA impurities in plasmid preparation?

XG Currently, different factors could impact residual host-cell RNA, such as molecular weight cutoff and membrane type. The type of membrane used can determine how efficiently residual host-cell impurities can be removed. For example, hydrophilic membranes are preferred as they can reduce unspecific binding. A high molecular weight cutoff is often favored, but this also presents challenges as estimations are based on the size of the plasmid. A conversion exists that helps determine molecular

weight cut-off based on the length of the plasmid. This is commonly used to determine the molecular weight cut-off for TFF I.

Q What was the pore size membrane used for TFF II?

XG As this is proprietary information, it is not possible to disclose the exact molecular weight. However, the molecular weight cutoff for a process can be calculated by using the values derived from the overall flux decay from TFF II. Molecular weight cutoff can be decided by screening different sizes of plasmid. In general, for final formulation, plasmid loss should not be a concern. At this stage, it is important to focus on maintaining a relatively high flux while also maintaining a consistent shear rate through the run, as the buffer composition can change over time.

Q What role does diafiltration buffer composition play in the TFF efficiency and plasmid quality, specifically from the impact of the rAAV complexation perspective?

XG Complexation efficiency can be impacted by pH from the final formulation buffer as well as high salt during the chromatography II process. The latter can also impact cell line bioactivity. To mitigate this, it is recommended to use a more neutral, low-salt condition for final buffer exchange.

Q In what ways has fully automating TFF I and II helped minimize batch-to-batch deviations and operator-dependent discrepancies in the downstream plasmid purification process?

XG To minimize discrepancies, it is necessary to monitor in-process parameters, such as weight, to accurately calculate the concentration factor. In some cases, the initial weight may not reflect the actual weight, potentially introducing errors into the process. A fully automated system addresses this by recording the actual weight after each step, which can then be used to update the concentration factor. This also allows the ability to alter calculations throughout the process without changing or modifying the existing platform.

LS One major improvement observed was a reduction in overall processing time. Steps related to TFF experienced the biggest reduction in time, including ultrafiltration/diafiltration (UF/DF) and concentration. These operations can now be fully automated, offering faster turnaround compared to benchtop, semi-automated systems. Manual systems, which depend on human intervention, are more susceptible to error. In such systems, products often remain idle or undergo prolonged recirculation, increasing more time in pump and shear. This can subsequently cause a lot of variation in the process. Automation allows valves and the recipe/protocol to adapt automatically without manual intervention, thereby preventing batch-to-batch variation.

Q How do you approach scaling from lab scale to pilot or GMP production in TFF?

XG When scaling up, particularly for cGMP manufacturing, it is essential to implement a Quality by Design (QbD) approach. This involves evaluating in-process parameters from small to large scale. In the case of TFF, a membrane surface area–based model is used. The principle is to treat the starting material as a function of the membrane's surface area, i.e., smaller membranes require a lower amount of plasmid. In other words, depending on the size of the membrane, a consistent ratio must be maintained between plasmid mass and membrane surface area. This is just one of the factors that need to be considered when scaling up.

Other factors that need to be considered include testing or validation of the process, not just at lab scale but also from a manufacturing perspective. The aim being to minimize manual operations across the workflow. Recording initial values at the beginning of each step is also important. Collectively, these measures will enable a smooth transition from lab-scale to large-scale manufacturing.

LS Traditionally, early process optimization is carried out at bench-scale on semi-automated systems. However, when it comes to scaling up, the process has to be moved to an entirely different system. As such, developers often have to return to the process development stage to determine what parameters and conditions are needed for large-scale production. In contrast, our KrosFlo RS TFF systems are purpose-built to enable scalability from bench to production. This ensures the conditions and parameters that are established at bench-scale are maintained upon scale-up at a manufacturing level.

BIOGRAPHIES

Xiangming Gu has been a pivotal figure in plasmid process development at Forge Biologics since 2023. Specializing in downstream process optimization and purification, his expertise in chromatography and tangential flow filtration has led to significant advancements in plasmid DNA processes. At Forge Biologics, Dr Xiangming is known for his dedication to achieving impactful results and fostering collaboration within the biopharmaceutical industry.

With decades of application engineering experience in biotech and medical device industries, **Lucas Smith** has consulted with companies globally on equipment design with a focus on bioreactors, mixers, and TFF systems. As product manager he plays a critical role in aiding the development and delivery of innovative TFF solutions by Repligen.

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AUTHORSHIP & CONFLICT OF INTEREST

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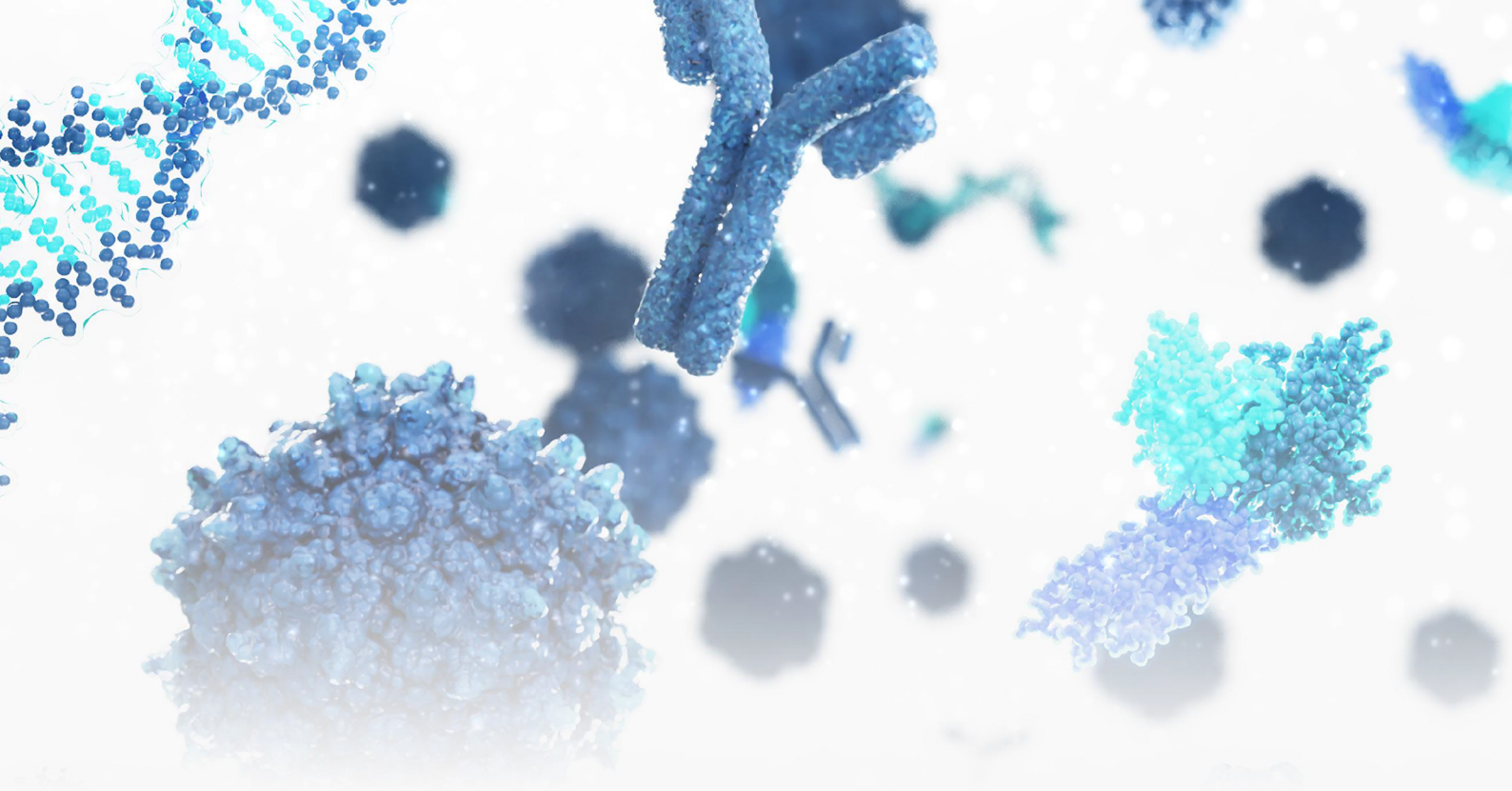

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CELL & GENE THERAPY INSIGHTS

Rethinking lentiviral manufacturing for cell and gene therapies: from platform design to point-of-care delivery

INTERVIEW

"The core challenge is that existing processes are often too expensive and too slow, creating major barriers to clinical entry and commercial success."

Lauren Coyle, Editor, BioInsights, speaks to Michael Kadan, Chief Operating Officer, Vector BioMed, and Boro Dropulić, Chief Executive Officer, Vector BioMed, on advances shaping lentiviral vector design and manufacturing. They discuss algorithm-driven vector engineering, targeted pseudotyping, and adaptable platforms that enhance transduction efficiency, scalability, and regulatory alignment. The interview highlights the growing momentum behind point-of-care manufacturing to reduce turnaround and expand access to autologous therapies. It emphasizes the need for streamlined phase-appropriate development and cost-efficient, platform-based solutions to sustain investment and growth across the cell and gene therapy sector.

Cell & Gene Therapy Insights 2025; 11(7), 909–917 · DOI: 10.18609/cgti.2025.101

Q From your perspective, what core elements define the 'next frontier' in lentiviral vector (LVV) design, development, and manufacturing, and how are they transforming the broader cell and gene therapy (CGT) landscape?

BD There are a few key elements, with the first being algorithm-based LVV design. A significant amount of effort is spent with our clients at the front end, focusing on vector optimization. A two- to three-fold improvement in titer or functionality can have a substantial impact by the time a product reaches commercialization. Designing the vector with the right elements, such as optimal promoters, transgene expression levels, and construct configuration, is critical. Investing in that design stage early on offers substantial benefits later.

Many CDMOs typically take a construct that has been developed in an academic laboratory and proceed directly to manufacturing. In contrast, we take a more specialized and collaborative approach by working closely with clients to optimize the vectors before they advance into clinical development. Multiple versions of a given vector are often produced to identify the one that achieves both high titer and optimal functionality, in alignment with the client's target product profile.

Another key element is vector targeting. Rather than relying exclusively on the standard vesicular stomatitis virus glycoprotein (VSVG) envelope, there is increasing interest in alternative pseudotypes that support targeted delivery. For example, envelopes such as cocal, show promise in both *ex vivo* and *in vivo* applications when combined with targeting motifs. These approaches may enable more selective transduction of specific cell types, including vectors tailored for T-cell and stem cell transduction, which may support more precise targeting strategies.

Together with advances in scalable and streamlined manufacturing, these innovations are helping to reduce costs, improve product quality, and support broader access. As gene therapy continues to mature, these technical refinements are shaping the next wave of LVV development.

Q Looking now at specialized CDMOs, what operational shifts have occurred, and what still needs to happen for CDMOs to move from industrialization to operationalization? What role does Vector BioMed play in that transformation?

MK In recent years, some CDMOs have approached CGTs as an extension of their existing service portfolios, generally built around monoclonal antibodies and therapeutic proteins. The assumption has often been that manufacturing

> "...experience has shown that CGT manufacturing demands a more specialized and responsive approach informed by product specific experience."
> Michael Kadan

Cell & Gene Therapy Insights 2025; 11(7), 909–917 · DOI: 10.18609/cgti.2025.101

processes, CGMP facilities and conventional quality systems used in biologics production would translate directly to CGT manufacturing. However, experience has shown that CGT manufacturing demands a more specialized and responsive approach informed by product specific experience.

This has led to several large, well-resourced CDMOs struggling to consistently deliver high-quality products in this space. It has highlighted the limitations of applying a traditional industrial model, one focused on automation, centralization, and scale, to a field that often requires flexibility, customization, and rapid iteration.

For CGT manufacturing to be operationalized effectively, CDMOs must focus on reducing concepts to practice. This means demonstrating proof of concept early, confirming manufacturability, and moving efficiently from vector design to clinical-grade production. Speed and adaptability are essential, particularly in the early stages when developers need to refine and test their constructs iteratively.

Vector BioMed emphasizes this type of early partnership model, working closely with clients to generate functional material rapidly, enabling clinical translation and positioning programs for long-term success. Supporting this kind of development pathway will be critical to expanding the impact of CGTs and accelerating their path to commercialization.

Q The CGT industry continues to struggle with affordability and accessibility. What specific bottlenecks do therapeutic developers face along the development pathway and in vector manufacturing, and what must be addressed to close this gap?

MK A particularly persistent bottleneck is the continued reliance on legacy practices that are not phase-appropriate for early-stage development. While regulatory frameworks increasingly emphasize data-driven, risk-based decision-making, operational approaches have not always kept pace with this mindset.

For example, let's look at the production of plasmids for LVVs. Historically, the industry followed a paradigm developed over 20 years ago, where developers would invest months

"By leveraging accumulated process data and minimizing unnecessary redundancies, the gap between innovation and patient access can be closed."
Boro Dropulić

and hundreds of thousands of dollars into master bacterial cell banks and large-scale plasmid manufacturing, steps more appropriate for a final therapeutic product than for enabling early clinical development. These legacy practices add substantial cost and time without necessarily improving safety or efficacy.

To close this gap, these outdated assumptions must be challenged, and a more flexible, risk-based approach that aligns with the current regulatory climate should be adopted. Encouragingly, more vendors are beginning to support these streamlined pathways. However, broader adoption across the manufacturing ecosystem is still needed.

BD Another key bottleneck lies in the lack of true platform processes for LVV production. Many existing manufacturing methods are outdated and require extensive customization, which balloons both cost and timelines.

Developing modular, platform-based solutions that can be applied across a variety of constructs is essential. These approaches reduce the need for revalidation and can significantly accelerate development while improving affordability. By leveraging accumulated process data and minimizing unnecessary redundancies, the gap between innovation and patient access can be closed.

Q How is the service model different between supporting point-of-care (PoC) versus centralized manufacturing? What levers should CDMOs be pulling on to gain speed-to-patient while maintaining quality and scalability?

BD Many of the currently approved autologous therapies, particularly in the CAR T-cell and stem cell therapy spaces, have demonstrated strong clinical efficacy. In these cases, PoC manufacturing may offer distinct advantages over centralized models.

Centralized manufacturing is well-suited to traditional pharmaceuticals that benefit from economies of scale, such as monoclonal antibodies. However, in autologous cell therapy, where the starting material is a patient's cells and the final product must be returned to that same individual, the centralized model introduces logistical and cost-related inefficiencies. PoC manufacturing, which takes place either onsite or near the treatment center, can streamline this process by reducing transportation time, simplifying coordination, and improving turnaround [1].

One of the major benefits of this approach is the potential to shorten vein-to-vein time. In some current systems, patients may wait 5–6 weeks to receive treatment, a timeline that is not viable for those with rapidly progressing diseases. PoC models could enable more timely delivery, with manufacturing occurring over the course of days, or potentially even within a single day in the future.

In addition to time savings, distributed production models may help alleviate some of the reimbursement and logistical hurdles that arise when therapies must cross regional or state boundaries. Smaller, regionally focused manufacturing sites can serve nearby hospitals more efficiently, and the infrastructure required is typically less complex than that of large, centralized facilities.

The long-term success of this model will depend on coordination across a broad ecosystem, including manufacturing partners, clinical centers, hospitals, and regulatory bodies. Reducing production costs and shortening the time to treatment with autologous cell therapy products will be key to supporting their broader access.

MK To support these kinds of distributed models, simplifying cell processing workflows is also important. Many existing autologous manufacturing procedures rely on automated systems that are costly and technically demanding. There are ongoing efforts across the field to develop approaches that are more straightforward, resource-efficient, and suitable for use in a range of clinical settings.

Skepticism around the viability of PoC models often centers on concerns surrounding technical readiness. However, there is precedent in clinical practice. For example, global data from 2018 shows that more than 1,800 teams across nearly 90 countries performed approximately 100,000 bone marrow transplants [2]. These are complex procedures that require stringent quality standards and multidisciplinary expertise.

If institutions already have the capacity to carry out bone marrow transplants, it stands to reason that with the right tools and training, they could support the manufacturing of autologous cell therapies. Building on this existing infrastructure, and adapting processes to be more accessible and streamlined, may help bring advanced therapies closer to the patient in a safe and scalable way.

Q You emphasized the importance of commercial-friendly solutions. How do customized vector solutions and standardized, off-the-shelf platforms balance innovation with global regulatory considerations and affordability?

BD Balancing innovation with commercial viability requires thoughtful design at every stage of development. From a vector manufacturing perspective, one of the key considerations is to ensure that solutions are economical and scalable across development phases. Custom vector solutions can be commercial-friendly when they are designed to be cost-effective at small scale while remaining compatible with full-scale clinical and commercial production.

It is also important that these systems allow for early optimization. By refining the vector at the outset, through improved titer and/or functionality, this supports a more efficient downstream process, enhancing the chances of success in the clinic and beyond.

Alongside tailored solutions, there is a growing role for standardized off-the-shelf vectors, particularly in spaces such as CAR T-cell therapy, where certain constructs, including some CARs, are now off-patent. For organizations that lack the infrastructure or resources to develop their own constructs, off-the-shelf options (or rapid CAR-T formats) can lower barriers to entry. These solutions can support both autologous and stem cell-based approaches, and when paired with compatible cell processing methods, can streamline translation.

From a regulatory standpoint, harmonization is paramount. Custom and off-the-shelf platforms alike must be developed with global compliance in mind, particularly as sponsors look to expand across multiple jurisdictions. That said, complexity does not always equate to reliability. In fact, highly automated systems can introduce long-term cost burdens due to proprietary materials and platform lock-in. By prioritizing simplicity and resilience, while preserving the option to automate later, developers can retain flexibility, reduce upfront costs, and focus on validating their therapeutic approach.

This balance between flexibility, cost-efficiency, and regulatory alignment is vital to ensure more equitable access to advanced therapies.

Q In the next 18–24 months, what are the most critical inflection points or opportunities you anticipate in CGT manufacturing, and what risks do we face if this operational and strategic shift doesn't happen?

MK From my perspective, one of the most important inflection points is the need to significantly improve affordability and broaden access to CGTs. We are already seeing major industry players stepping back from their advanced therapy portfolios, partly because the field has not yet shown scalable, economically sustainable models.

The traditional high-cost, low-volume model is not viable in the long term. Instead, we need to reduce costs and expand access, enabling the treatment of more patients at lower margins. That shift could support both public health goals and long-term commercial sustainability. If we fail to make that adjustment, if the industry cannot show that it can deliver therapies at scale and at a reasonable cost, we risk a continued decline in investment and a contraction of opportunity across the sector.

BD Additionally, this is exactly why we have focused so heavily on optimizing every unit operation, from upstream to downstream, to increase efficiency and reduce costs. If these therapies remain unaffordable or cannot be reimbursed, investment in the space will continue to decline.

The core challenge is that existing processes are often too expensive and too slow, creating major barriers to clinical entry and commercial success. Unless the field addresses these bottlenecks, many promising therapies may never reach patients. Tackling this issue ensures that innovation can continue and that CGTs deliver their full potential.

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BIOGRAPHIES

Mike Kadan received his PhD in Biology from the Johns Hopkins University and later obtained an MBA from Frostburg State University, Frostburg, MD, USA. He began his biotech industry career in 1989 with one of the founding companies in gene therapy, Genetic Therapy Inc. As Chief Operating Officer, Mike brings over 30 years of experience in the development and manufacturing of biologics. He has acquired an in-depth understanding of the CMC related issues important for biologics product development and has contributed to numerous IND's, enabling the clinical evaluation of products ranging from retrovirus, adenovirus, monoclonal antibodies, and lentiviral vectors. During his career, Mike has held leadership roles responsible for a wide range of functions, including process development, manufacturing, CMO management, materials management, inventory control, warehouse operations, and logistics. In addition, Mike has participated in several capital projects for manufacturing facility design, build and commissioning. Mike's recent experience includes five years specializing in lentiviral vector scale up and manufacturing technology. As Director of Manufacturing at Lentigen Technology Inc. (a Miltenyi company) he led the implementation of a state-of-the-art platform process for lentivirus production and oversaw the successful completion of hundreds of GMP batches of lentiviral vector destined for human clinical trials.

Mike Kadan PhD MBA, Chief Operating Officer, Vector BioMed, Gaithersburg, MD, USA

Boro Dropulić received his PhD from the University of Western Australia, Crawley, Australia and his MBA from the Johns Hopkins University (JHU), Baltimore, MD, USA. He has been in the gene therapy field since the late 1980s. As Chief Executive Officer, Boro brings over 30 years of leadership and experience in the design, development, manufacturing, clinical translation, regulatory, clinical implementation, and commercialization of lentiviral vector technology. After a Fogarty Fellowship at the NIH, he joined the faculty at JHU where he worked on developing lentiviral vectors as delivery systems for gene therapy. After 4 years in academia, he founded his first company ViRxSys and led the team that first demonstrated the safety of lentiviral vectors in humans with his UPenn colleagues. Later he founded Lentigen, which first developed the Lentiviral vector used to produce Kymriah®, the first FDA-approved gene therapy product. He implemented and directed the company's CDMO business model and therapeutic pipeline of gene therapy products. He led Lentigen until 2021, and then left to co-found and launch Caring Cross, a 501 (c)(3) non-profit, and serves as the Executive Director. Lentiviral vectors are critical to produce many gene therapy products such as CAR-T cells and gene-modified hematopoietic stem cells (HSCs) for the treatment of an increasing number of important diseases. Presently there are huge bottlenecks in obtaining high quality lentiviral vectors in a reasonable time that motivates investigators and investors alike. This delays and puts at risk the development and commercialization of innovative and potentially curative gene therapies that are desperately needed. Seeing a need to help investigators improve and accelerate their medicinal concepts needing lentiviral vectors into the clinic, Vector BioMed was launched and Boro serves as the Chief Executive Officer.

Boro Dropulić PhD MBA, Chief Executive Officer, Vector BioMed, Gaithersburg, MD, USA

AUTHORSHIP & CONFLICT OF INTEREST

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

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Optimizing for faster quality control in cell therapies:
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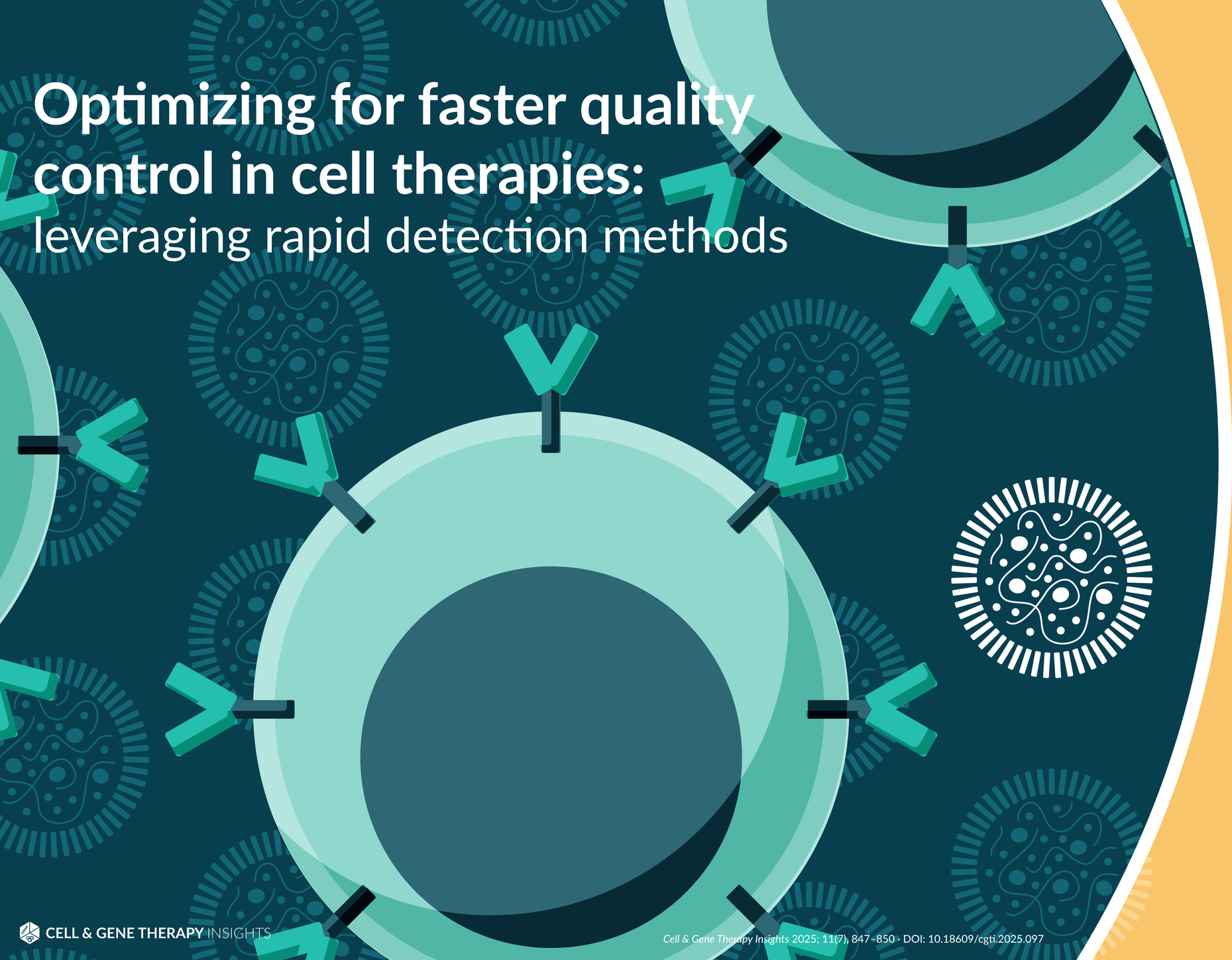

CELL & GENE THERAPY INSIGHTS

Cell & Gene Therapy Insights 2025; 11(7), 847–850 · DOI: 10.18609/cgti.2025.097

Optimizing for faster quality control in cell therapies: leveraging rapid detection methods

Advancing mycoplasma detection and sterility testing in cell therapy manufacturing

Cell therapy manufacturing requires precise biological processes and advanced analytics to ensure the safety, potency, and quality of the therapeutics. Traditional compendial assays for bacterial contamination testing, such as *USP <63>* and EP 2.6.7, rely on culture or PCR methods, but culture-based tests are slow and may miss non-culturable species. Additionally, compendial sterility testing methods are challenged by limited sample volume, short shelf-life, and long turnaround times, highlighting the need for novel technologies.

Rapid PCR-based platforms, such as the Applied Biosystems™ MycoSEQ™ Plus Mycoplasma Detection System and the SteriSEQ™ Rapid Sterility Testing Kit, help enable rapid, sensitive mycoplasma testing and bacteria/fungi detection for in-process, raw material, and for mycoplasma testing lot release, delivering results in under 5 h.

The MycoSEQ Plus assay detects under 10 copies/reaction, covers over 200 Mycoplasma species, and minimizes cross-reactivity, while the SteriSEQ assay uses primers designed for the 16S and 18S rRNA regions, detects as low as 5 GC/reaction, and covers over 16,000 bacteria and 2,600 fungi species.

Meet the expert

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Case Studies

Case study 1: Evaluating the sensitivity and specificity of MycoSEQ Plus

In an internal study, 10 genome copies (GC)/mL gDNA of three species (*M. arginini*, *A. laidlawii*, and *U. urealyticum*) were spiked into the sample matrix and processed using a standard lot release workflow. As shown in **Figure 1A**, Ct values for T cell spent media spiked with these species were all below the 38 cut-off. Similarly, Chinese Hamster Ovary (CHO) bulk harvest media spiked with 16 Mycoplasma species and detection controls **(Figure 1B)** also tested positive with Ct values under 38. These results demonstrate the assay's robustness in detecting Mycoplasma across diverse sample types and conditions.

Additionally, specificity testing against closely related bacteria and common bioprocess materials (e.g., CHO DNA, human genomic DNA, kanamycin) showed no false positives, confirming the assay's high specificity.

Key takeaway

Case Studies

Evaluating the sensitivity and specificity of MycoSEQ Plus see more...

Rapid detection of bacteria and fungi with the SteriSEQ assay see more...

Assessing the SteriSEQ assay workflow on CAR-T cell samples see more...

Case study 2: Rapid detection of bacteria and fungi with the SteriSEQ assay

In another study, bacterial and fungal species were evaluated using the SteriSEQ rapid detection assay workflow.

As shown in **Figure 2A**, gDNA of USP <71> bacterial species with DNA titers ranging from 5–25 CFU equivalents were spiked into samples, extracted, and tested using the SteriSEQ assay workflow. Results demonstrate that the assay detects below the USP guideline limit of 10-100 CFU, confirming its high sensitivity and suitability for sterility testing. Similarly, the system detected fungi species in 10^6 Jurkat cells. The data illustrated in **Figure 2B** highlights the detection of two fungal species at 5 GC/reaction: both show strong signals with minimal background, demonstrating the assay's sensitivity and specificity for fungal detection.

Key takeaway

Case Studies

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Case study 3: Assessing the SteriSEQ assay workflow on CAR-T cell samples

In another study, bacterial and fungal species were evaluated using the SteriSEQ rapid detection assay workflow.

In another study, the SteriSEQ rapid sterility testing kit workflow was tested on CAR-T cells (1–1.5 × 10^6 cells, ~15–20 ng DNA/well). As shown in **Figure 3**, all samples and controls showed expected signals: the internal positive control (IPC) must be positive, DPC samples positive for all dyes, and unknown negative samples only positive for IPC. Trials 1 and 2 showed negative samples as expected, but Trial 3 revealed two bacterial positives despite sterile cultures and good viability. Three days later, contamination became visible in the culture as well.

Key takeaway

Summary

"MycoSEQ Plus and SteriSEQ rapid sterility assays deliver same-day actionable results from a variety of sample types. These assays are aligned with current regulatory guidelines, including *USP <63>* and *USP <71>*, respectively. Lastly, they are straightforward and proven qPCR workflows with protocols that include sample preparation, assay setup, and result analysis with software designed for GMP testing."

Q&A

Q How can we integrate Mycoplasma and sterility testing into the cell therapy manufacturing workflow without compromising the viability and functionality of the final product?

Q How do the regulatory agencies view nucleic acid testing for sterility testing?

Q How does SteriSEQ differentiate itself from other sterility testing methods available on the market for cell therapy products?