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SPOTLIGHT

Delivery of the future

CELL & GENE THERAPY INSIGHTS

CONTENTS VOLUME 11 · ISSUE 11

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CELL & GENE THERAPY INSIGHTS

DELIVERY OF THE FUTURE

SPOTLIGHT

Redefining targeted AAV delivery

Benjamien Moeyaert, Lolita Petit, and Els Henckaerts

VIEWPOINT

"The convergence of capsid engineering, conjugation chemistry, and ligand design is opening new avenues for truly targeted gene delivery."

The maturation of AAV-based gene therapy has intensified demand for vectors with both high potency and precise, cell-specific delivery, yet traditional capsid engineering approaches remain limited by manufacturability, translational gaps, and slow development. Emerging post-manufacture conjugation platforms, enabled by advanced chemical and enzymatic strategies, offer a modular alternative by attaching targeting ligands to purified AAV capsids, improving flexibility, scalability, and translational relevance.

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Over the past two decades, the field of AAV-based gene therapy has achieved monumental success, moving life-changing and life-saving treatments from the lab bench to market [1]. Nevertheless, the long-term success of AAV platforms is constrained by the difficulty of ensuring both high vector potency and accurate, cell-specific delivery.

Relying on naturally occurring AAV serotypes or even first-generation engineered capsids often results in off-target biodistribution—a problem most prominent after intravenous administration—which risks unwanted side effects [2].

The scientific community's response has been an intensive focus on capsid engineering, with techniques such as directed evolution delivering impressive results [3]. By inserting peptide sequences into surface-exposed loops and selecting for performance *in vitro* and *in vivo*, researchers have developed capsids with enhanced tropism for tissues such as liver, muscle, and the central nervous system (CNS) [4]. These developments have benefited from machine-learning guided library design, high-throughput screening platforms, and advances in sequencing methods [5].

However, we have observed that the seamless translation of these engineered capsids into clinical reality is often blocked by critical CMC challenges. Engineered capsids frequently exhibit poor yield during large-scale manufacturing, thus requiring bespoke process development and ultimately contradicting the fundamental requirement for a robust and reproducible GMP process [6]. Directed evolution is also inherently slow and resource-intensive, with limited transferability across serotypes or new disease contexts. Every new targeting goal requires a completely new vector, frustrating efforts to standardize the up- and downstream manufacturing processes. Moreover, capsid design and selection in rodent or non-human primate models often fails to predict human clinical translation, forcing costly and time-consuming 'redesign'. Rational design approaches, whereby targeting groups such as peptides, DARPins or antibody fragments are genetically inserted into the capsid structure, relieve some of these issues [7]. However, they continue to face constraints, as the AAV capsid structure imposes limits on the inserted ligand size, presentation, and number, and even small modifications can hamper its stability and manufacturability.

An alternative is emerging that resolves these industrial constraints by enforcing a conceptual and physical decoupling of the vector manufacturing and the targeting function: post-manufacture conjugation of targeting ligands to AAV capsids. This strategy, inspired by targeted drug conjugates [8], involves the attachment of targeting groups onto the surface of a purified AAV vector. This progress is driven by the development and maturation of AAV-specific conjugation technologies. Leading industry players, including Coave Therapeutics with its ALIGATER™ platform, have pioneered efficient chemical conjugation techniques that enable efficient linkage of a wide variety of targeting moieties to the capsid [9,10]. In parallel, Tavira Therapeutics recently developed an advanced enzymatic conjugation strategy offering effective and highly specific conjugation in a fully proteinaceous system [11]. Moreover, both platforms start from capsids featuring pronounced liver de-targeting, contributing to an improved safety profile. Beyond these specific technologies, other innovations continue to expand the space with alternative chemical [12] or enzymatic methods, and even non-covalent systems are maturing rapidly.

Much of the early work is focusing on CNS delivery and transduction, where validated ligands and receptor biology provide a rich foundation to demonstrate the potential of conjugation [13]. We envision that as the biology behind targeted delivery of (bio) pharmaceuticals becomes more defined, the toolbox of targeting groups for specific tissues and cell types will expand. In combination with these fully matured conjugation technologies, this approach will undoubtedly lead to well-characterized, high-specificity, high-potency capsids for a broad range of indications.

These developments represent a fundamental conceptual shift in capsid engineering strategy. Rather than embedding

the targeting information genetically within the capsid structure, we can now modularly append it after production. This offers several advantages. First, it allows for the use of validated human-specific ligands, improving translational relevance. Second, it gives far greater flexibility in ligand design, presentation, and affinity maturation as it decouples ligand screening from capsid engineering. This enables quick deployment of any validated ligand to multiple well-characterized capsids with favorable properties such as immune evasion or liver de-targeting. Crucially, it also allows for the production and validation of a single, common AAV intermediate at commercial scale. This modularity simplifies manufacturing, accelerates iterations, and allows the industry to focus resources on optimizing a single robust manufacturing platform. Collectively, these features establish a streamlined platform that drastically accelerates development timelines, reducing R&D associated costs and shortening the path to clinical translation. It also opens the door to disease-reacting ligands and/or ligands with immune masking or immunotolerance functions.

Of course, no advanced platform is without its challenges. Reproducibility, scalability, and robust analytical characterization require careful attention throughout the design process, a necessity compounded by the fact that regulatory frameworks for conjugated biopharmaceuticals are still evolving.

Nonetheless, the path forward is clear. The convergence of capsid engineering, conjugation chemistry, and ligand design is opening new avenues for truly targeted gene delivery. Emerging companies—including Tavira Therapeutics and Coave Therapeutics—are advancing novel conjugation platforms and therapeutic programs, creating a robust ecosystem that established players are now leveraging to augment their pipelines. The convergence of capsid engineering, conjugation, and ligand design is re-shaping what is possible for targeted gene delivery.

In summary, the field of AAV conjugation is rapidly maturing, not as a replacement for traditional capsid engineering technologies, but as a powerful expansion of the toolkit. It offers a complementary path towards truly targeted gene therapy—one that is modular, scalable, and increasingly clinically viable. By embracing this strategic shift towards post-manufacturing customization, we move beyond the limitations of current AAV-based gene delivery solutions and bring the promise of gene therapy closer to reality for a wider range of patients and diseases.

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BIOGRAPHY

Benjamien Moeyaert is a Research Manager at the Trellis research group at KU Leuven. He earned his PhD in Biochemistry and Biotechnology from KU Leuven, where he focused on protein engineering and super-resolution fluorescence microscopy. He subsequently completed a postdoctoral fellowship at the HHMI Janelia Research Campus in Virginia, USA, advancing fluorescent protein–based biosensors for neuroscience applications. After returning to KU Leuven, he joined the Trellis research group, where he leads and manages multiple innovation-driven projects in the AAV gene therapy field. His work on enzymatic conjugation of AAV vectors forms the foundational technology of Tavira Therapeutics, a KU Leuven spin-off, where he serves as co-founder.

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Lolita Petit is a genetic medicine expert with over a decade of experience in gene therapy across pharma and biotech. Her expertise spans the full R&D continuum from novel target and vector concepts to the translation and advancement of gene therapy candidates toward the clinic. Lolita joined Coave Therapeutics in 2023, where she now leads the company's scientific strategy, the development of its next-generation conjugated AAV platform and therapeutic programs. In her role, she oversees platform activities, payload and AAV engineering, preclinical, translational, and IP operations to drive Coave's pipeline toward FIH studies. Previously at Janssen Pharmaceuticals (J&J), she managed the Translational Gene Therapy Research Team responsible for the selection and characterization of gene therapy candidates across multiple therapeutic areas, while also directing new delivery and immunomodulation platform efforts for ocular gene therapy applications. Prior to J&J, she worked at Spark Therapeutics (Roche) where she led the Ocular platform team and oversaw the optimization of innovative gene therapy vectors and novel gene therapy strategies. Lolita holds a PhD in Biotechnologies and Therapeutics from the University of Nantes, Nantes, France.

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Els Henckaerts leads the Trellis research group at KU Leuven. She earned her medical degree from the University of Antwerp and completed her doctoral studies in stem cell biology through a joint program between the University of Antwerp, Belgium, and the Icahn School of Medicine at Mount Sinai (MSSM) in New York City. Remaining at MSSM for her postdoctoral research, Els shifted her focus to virology, specifically investigating virus-host interactions in the context of adeno-associated virus (AAV). She continued working on this topic and established her own research group at King's College London where she broadened her research to include AAV-mediated gene therapy. This expansion sparked fruitful collaborations with both biotech and pharmaceutical partners. In 2019, Els returned to Belgium to establish the Trellis research group and co-founded Handl Therapeutics. Trellis is dedicated to advancing various aspects of AAV gene therapy, ranging from pivotal IND enabling studies to pioneering innovations in gene delivery and manufacturing. In 2023, Trellis and KU Leuven created the spin-off Tavira Therapeutics, a cutting-edge biotech startup leveraging a next-generation capsid platform for precise gene delivery.

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CELL & GENE THERAPY INSIGHTS

DELIVERY OF THE FUTURE

SPOTLIGHT

Beyond the liver: reimagining delivery systems for the future of gene and RNA medicines

Paloma Giangrande

VIEWPOINT

"Expanding genetic medicine beyond the liver will require more than better materials—it will require collaboration without boundaries."

Over the past decade, gene and RNA therapeutics have moved from scientific promise to clinical impact—transforming once-hypothetical interventions into life-changing realities. Yet a persistent challenge continues to define the field: delivery [1]. The liver has served as both a proving ground and a safe harbor for innovation, thanks to its natural receptivity to lipid nanoparticles (LNPs) and AAV vectors [2]. More recently, N-acetylgalactosamine (GalNAc) conjugates have further expanded the liver's therapeutic potential by enabling highly specific, receptor-mediated delivery to hepatocytes via the asialoglycoprotein receptor (ASGPR) [3]. But most diseases don't begin in the liver—and the next frontier in genetic medicine will depend on our ability to reach far beyond it (**Figure 1**).

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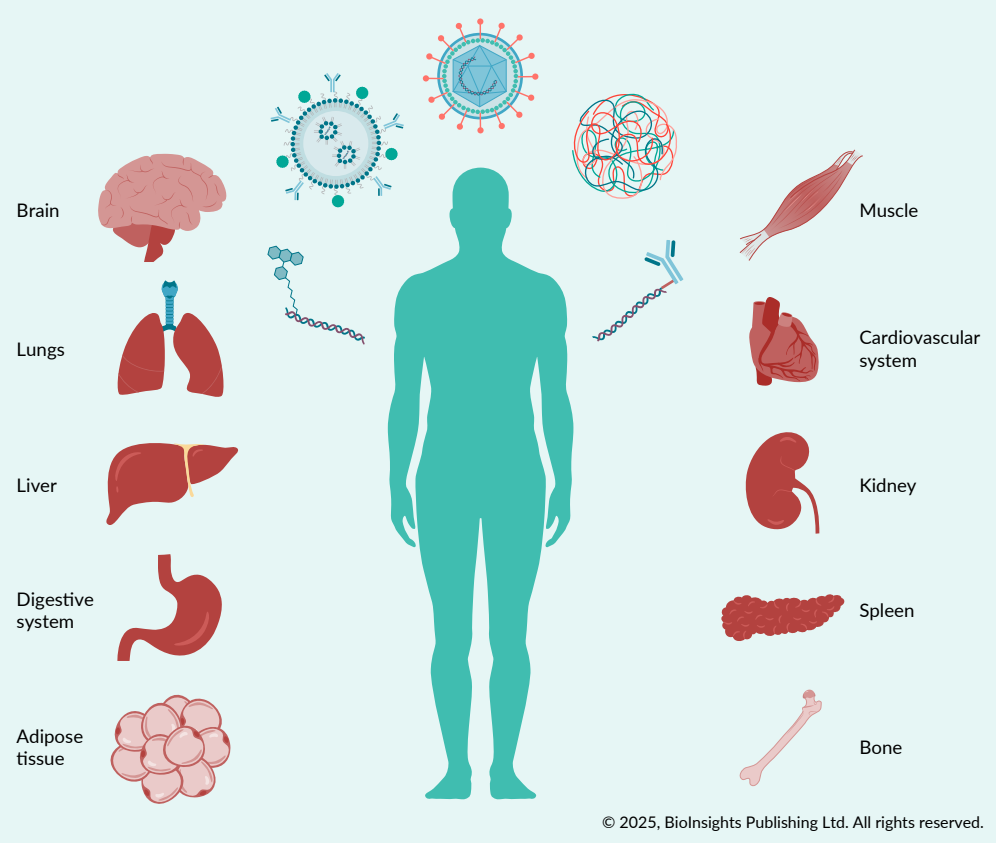
FIGURE 1

Expanding the reach of genetic medicines: strategies for extrahepatic delivery.



This figure illustrates emerging delivery technologies designed to overcome hepatic tropism and enable targeted gene and RNA therapeutic delivery to extrahepatic tissues. Key innovations include: engineered AAV capsids developed via directed-evolution to enhance tropism for muscle, lung, and central nervous system; lipid and polymeric nanoparticles (LNPs/PNPs) functionalized with tissue-specific ligands, guided by single-cell transcriptomic and proteomic datasets; biomimetic carriers that emulate endogenous transport systems such as exosomes and lipoproteins to improve cellular uptake and biodistribution; and novel conjugates analogous to GalNAc, tailored for selective uptake in kidney, spleen, and immune cell subsets via receptor-ligand interactions. Together, these approaches represent a systems-level integration of material science, molecular targeting, and computational design—paving the way for precision delivery beyond the liver.

THE LIVER WAS ONLY THE BEGINNING

Therapies targeting hepatic disorders, from familial hypercholesterolemia to transthyretin amyloidosis, have validated the promise of both nucleic acid therapeutics and gene replacement technologies [2,3]. The liver's unique architecture and active uptake/endocytosis made it the logical first target for LNP- and AAV-based systems [2]. For smaller therapeutic payloads, GalNAc-mediated delivery has harnessed ASGPR to enable highly selective uptake by hepatocytes, further expanding the liver's role as a gateway for precision therapeutics [3].

However, this initial success has created an unintentional boundary. The same hepatic tropism that enabled these early breakthroughs now limits our reach to the heart, lungs, muscles, pancreas, adipose tissue, and brain—where many of the most challenging and urgent therapeutic

needs remain [4]. Indeed, for diseases like Duchenne muscular dystrophy, cystic fibrosis, or neurodegenerative disorders, the inability to deliver payloads effectively beyond the liver is slowing progress where it's needed most.

TARGETING WITH PRECISION

Reaching non-hepatic tissues requires mastering the art of tropism. Systemic barriers—tight endothelial junctions, specialized clearance mechanisms, and immune detection—can thwart even the most sophisticated delivery vehicles. But progress is accelerating.

Directed evolution is yielding novel AAV serotypes with enhanced affinity for muscle, lung, and central nervous system tissues [5]. In parallel, LNPs decorated with targeting peptides or receptor-binding ligands are being screened for tissue-specific uptake using data derived from single-cell transcriptomes and proteomics [6]. Mimicking biological transport systems—such as designing nanoparticles that emulate lipoproteins or exosomes—is another creative route to access hard-to-reach cells [7]. Complementing these efforts, researchers are actively identifying conjugates—akin to GalNAc—for extrahepatic delivery, leveraging receptor-ligand biology to enable selective uptake in tissues such as kidney, spleen, and immune cell subsets [8].

BREAKING THE PAYLOAD BARRIER

Delivery systems remain constrained not only by where they can go, but by how much they can carry. For gene therapy indications, AAVs remain favored for their safety and clinical validation, yet their modest capacity of roughly 4.7 kilobases restricts applications requiring larger transgenes or complex gene-editing systems such as CRISPR-Cas variants [9].

Non-viral alternatives such as LNPs and polymeric nanoparticles (PNPs) offer flexible payload design, but trade-offs between stability, size, and biodistribution persist [10]. Indeed, larger LNP or PNP particles tend to aggregate or get cleared too quickly [11]. To overcome this, researchers are pushing into new territory—using machine learning to design AAV capsids with expanded capacity, hybrid nanoparticle systems that self-assemble dynamically, and modular encapsulation architectures capable of carrying multiple components safely [12]. At the same time, engineers are reimagining the payload itself—employing compact coding sequences and split-intein systems that make efficient use of limited space [13].

A SYSTEMS APPROACH TO DELIVERY

The future of therapeutic delivery depends on closing the gap between vehicle and payload engineering. Instead of viewing them as separate challenges, researchers are increasingly integrating them—optimizing endosomal escape, tuning lipid ionization states, and designing biodegradable polymers for controlled release. Fine-tuning immune compatibility and circulating half-life now involves as much chemistry and computation as biology.

Computational modeling, AI-guided material discovery, and *in silico* simulations are transforming how we explore the design space, reducing empirical trial-and-error and empowering rational, data-driven iteration [14][15].

THE NEXT FRONTIER: INTEGRATION & COLLABORATION

Expanding genetic medicine beyond the liver will require more than better materials—it will require collaboration without boundaries. The convergence of

material science, structural biology, AI, medicinal chemistry, and clinical pharmacology is essential to unlocking this next phase. Regulatory science must evolve too, recognizing the hybrid nature of these emerging delivery constructs that sit between biologics, devices, and digital models.

Ultimately, the story of gene and RNA medicine is one of precision—not just in editing or expression, but in where therapies go. The breakthroughs of the last decade showed us what happens when we reach the right target. The breakthroughs of the next will show us what becomes possible when we can choose any target at all.

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BIOGRAPHY

Paloma Giangrande is a leading expert in nucleic acid therapeutics and RNA delivery. She pioneered aptamer-guided siRNA delivery beyond the liver, expanding the reach of RNA interference. Her academic work laid the foundation for multiple RNA platforms, and she has since held senior roles at Moderna, Wave Life Sciences, and Eleven Therapeutics, driving programs in mRNA, RNA editing, and xRNA technologies. Currently, she serves as SVP of Platform and Translational Sciences at Orbital Therapeutics and Editor-in-Chief of *Molecular Therapy—Nucleic Acids*. With over 70 publications and 10 patents, she continues to shape the future of RNA-based medicine through strategic leadership and scientific innovation.

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CELL & GENE THERAPY INSIGHTS

DELIVERY OF THE FUTURE

SPOTLIGHT

Vector vision: advancing viral design, AI-driven precision, and global access in gene therapy

INTERVIEW

"*In vivo* is the big win here. If we can demonstrate that *in vivo* delivery works safely and effectively—as early trials such as Interius in Australia are beginning to show—it could open up a new era."

Abi Pinchbeck (Editor, *Cell & Gene Therapy Insights*) speaks with Semih Tareen (Independent Consultant) about the evolving landscape of viral and non-viral delivery, AI's role in advancing gene therapy, and the future of *in vivo* engineering.

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Q What are you working on right now?

ST I was at Sana Biotechnology until the end of 2023, and I have been following their clinical progress with great interest, especially in type I diabetes. My team and I were involved in many of those pipelines, particularly those focused on gene delivery using viral vectors.

At the moment, I am spending my time between Seattle and Istanbul. I am exploring whether it might be possible to help establish a cell or gene therapy capability in

"When safe integration is essential, viral vectors derived from retroviruses and particularly lentiviruses will continue to have an advantage, as long as promoter design is carefully considered."

this part of the world. Turkey has a strong industrial foundation, including in medicine, and attracts many visitors for medical tourism, though most of that is focused on cosmetic treatments. I am curious about whether this foundation could also be leveraged to make advanced therapies like CAR-T available locally, as there are currently no approved CAR-T therapies in Turkey or the surrounding region, and there is a high unmet need in this part of the world.

In the meantime, I am also running a biotech consulting practice, providing guidance to companies on gene delivery needs. I am always open to connecting with new clients interested in these areas.

Q Viral vectors have long been the workhorses of gene delivery. How do you see their design space evolving in response to the surge of non-viral approaches?

ST Even though I am a virologist at heart, I believe both viral and non-viral platforms have important roles to play. If non-viral vectors overcome some of the challenges of viral vectors, that would be phenomenal. There are pros and cons to both approaches. For therapies such as CAR-T, where persistence is key, meaning we want the cell product to survive, replicate, and continue functioning, integrated gene delivery systems may be important for ensuring persistent gene expression through cell proliferation.

We can work with non-viral tools that integrate, such as transposons, but viral vectors, particularly retroviruses and lentiviruses derived from HIV, have evolved to integrate successfully. When safe integration is essential, viral vectors derived from retroviruses and particularly lentiviruses will continue to have an advantage, as long as promoter design is carefully considered.

Non-viral systems, especially after the COVID-19 vaccines, have shown that they can be manufactured and delivered safely. My specialty is also in overcoming antiviral resistance in cells. Once viral vectors enter a cell, they can trigger intracellular sensors. The COVID vaccines would not have been possible without the Nobel Prize-winning work of Drew Weissman and Katalin Karikó on overcoming these intracellular defense mechanisms.

Viruses evolved to do this naturally, while non-viral systems rely on chemical modifications such as pseudouridine used in mRNA vaccines to overcome those defenses. So, even putting my bias aside, viral vectors may still have an edge when it comes to functionality. That said, non-viral systems are likely more affordable to produce, especially since viral vector manufacturing involves higher costs, particularly costly safety testing and analytics for detecting replication-competent viruses. Non-viral systems, on the other hand, still require a delivery tool. While lipid nanoparticles have been widely used other approaches such as electroporation also add costly steps to gene delivery.

Each platform has its strengths and limitations, and I think both will continue to thrive depending on the application.

Q You've worked at the frontier of CAR-T development. How have advances in gene delivery vector engineering changed the scope/scalability of what's possible in next-generation cell therapy manufacturing?

ST The viral vector itself honestly has not changed much over the years in terms of engineering; most companies still use the standard assortment of AAVs, adenoviruses, or third generation lentiviral vectors. However, there have been some revolutions that have changed how we think about overcoming barriers to gene delivery.

The typical approach to maximizing viral vector efficiency has been to maximize titers in production, but a less appreciated way to increase output is to enhance the infective dose or transduction units of the vectors themselves. One way to accomplish this is by advancing vector design. Back in 2014, when I was at Immune Design, we started incorporating new vector engineering tools to enhance titers not in production, but at the transduction stage, making the vector more infectious by helping it overcome cellular barriers. While at Sana Biotechnology we explored overcoming similar cellular barriers chemically in addition to biologically. All this work has been published and/or presented. This kind of R&D in vector engineering will be a big win for the field.

Another area is promoter design. Many programs use standard viral promoters, but these can raise safety issues due to enhancer activity and insertional activation risks. Cellular promoters are proving safer in that respect: they don't appear to show the enhancer activity present in viral promoters, thus less of a chance for insertional oncogenesis. Therefore, designing better promoters for enhanced safety will be another key direction.

Q Last year, you wrote a piece for *Cell and Gene Therapy Insights* about the shift from *ex vivo* to *in vivo* gene engineered cell therapies. How do you see advances in vector design and AI-driven prediction combining to make direct *in vivo* delivery safer and more precise?

ST I am very hopeful and excited about *in vivo*, especially the design of tropism. When I was at Juno Therapeutics, we were still working with *ex vivo* platforms. HIV, for example, can infect T cells very effectively, and even then I was thinking about whether viral vectors modeled on HIV could target T cells *in vivo* for in-body CAR-T production.

Today, companies such as Sana Biotechnology and Umoja are doing just that. Until now, we have largely relied on empirical reasoning—trial and error—to design vector envelopes. I would love to see AI applied to reconfigure that landscape. Researchers like David Baker and his colleagues in Seattle are already using computational approaches

and protein cages to achieve functional target-driven tropism. AI will be an invaluable tool for that kind of design.

Q I know you've spoken previously about AI tools—can you comment on the evolving role that AI and data science are playing in cancer immunotherapy, specifically thinking about delivery tools?

ST AI is not my area of expertise, but I enjoy discussing it. In CAR-T, one fascinating question is why some cells persist in the patient while others do not. When they do persist, it tends to be certain subpopulations of cells. AI could help us identify what makes those cells special and engineer all these cells to behave in that way, so they persist and are effective without causing toxicity.

AI could also support clinicians in managing side effects such as neurotoxicity and cytokine release syndrome, which sometimes require the management of complicated symptoms in intensive care. Beyond that, AI could help improve promoter and enhancer design for safer gene delivery.

Q More specifically, how do you see AI reshaping the dialogue between regulators and developers, such as in validating predictive models in advanced gene therapy programs?

ST It would be great if AI could predict certain behaviors and outcomes, but so far, AI is only as good as the data it is trained on. I do not think it will replace animal or human data anytime soon, we will still need those studies, but it could save time and cost in some areas. If AI can help us gain back a few months, that would be huge for patients.

For example, the replication-competent lentivirus assay is expensive and requires setting aside vector product for testing. I wonder if AI could help in dialogue with regulators in accepting certain modeled data. Furthermore, if AI could reliably predict integration sites and their safety profiles, that could be another valuable tool in regulatory discussions.

Q Looking ahead five to ten years, what innovations do you believe will redefine the boundaries of what viral delivery systems can achieve/how do you see the space evolving?

ST *In vivo* is the big win here. If we can demonstrate that *in vivo* delivery works safely and effectively—as early trials such as Interius in Australia are beginning to show—it could open up a new era. I do not think it will replace *ex vivo* therapy, but it will expand possibilities enormously. There will be regulatory and safety challenges, but those can be overcome. This could be the next major paradigm shift, similar to the beginnings of gene therapy.

Q Personally, what are your key goals and priorities in your own work for the next 1–2 years?

ST If people who currently do not have access to some of these already approved therapies like CAR-T in certain parts of the world could gain access, I would love to be part of helping to make that happen. For example, our friends in the US at Caring Cross are doing a lot of great work with governments in other countries, such as Brazil, to help bring CAR-T cell therapies to patients there.

For me, the opportunity would be in Istanbul and this part of the world, to see if something similar could be possible. I am trying to understand the investment climate here in Turkey, and it is quite different from that in the US. However, the investment does not need to come from within Turkey.

I would welcome anyone to reach out to me on LinkedIn if they have ideas, suggestions, or would like to be part of this effort.

BIOGRAPHY

Semih Tareen is a biotech team leader and molecular biologist who specializes in developing gene delivery tools for biotechnology companies. He and his teams have enabled cutting edge preclinical gene and cell therapy startups to reach their clinical milestones and approvals.

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CELL & GENE THERAPY INSIGHTS

DELIVERY OF THE FUTURE

SPOTLIGHT

Building scalable, science-forward gene delivery platforms

INTERVIEW

"Ultimately, the success of next-generation delivery technologies will depend on how well we align performance with real-world deployability."

Abi Pinchbeck (Editor, *Cell & Gene Therapy Insights*) speaks with Brenna Kelley-Clarke (Independent Consultant) about emerging paradigms in gene delivery, the evolving relationship between viral and non-viral systems, and the analytical and regulatory frameworks shaping the field's future.

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Q What are you currently working on?

BK-C Earlier this year, I was involved in an industry–academic collaboration as a consultant. The goal is to generate an open-access platform for AAV manufacturing and testing.

There are a number of ultra-rare genetic diseases that are great candidates for AAV-based gene therapy. However, the cost of developing the AAV vector, the manufacturing process, and all the associated analytical tools from scratch is often cost prohibitive, especially for small companies or academic institutions, and particularly when we are talking about such small patient populations.

"Companies are looking for delivery technologies and manufacturing strategies that will support scalability, broader patient access, and more off-the-shelf solutions."

The goal of the project is to create a knowledge base that will enable teams to jump-start the development of these AAV gene therapies for ultra-rare genetic diseases.

Q You've worked across both viral and non-viral systems through major inflection points in the field. How would you describe the most significant shifts in the gene delivery landscape over the past decade, and what forces are driving those changes today?

BK-C The last decade has seen an exciting shift from proof of concept into real-world therapeutic success for various gene delivery modalities. Top of mind, for example, would be CAR-T cell therapy as well as AAV-based gene and cell therapies that have both demonstrated curative potential. Now we are also seeing gene editing therapeutics enter clinical trials.

Alongside these successes, we have also had a major reality check. Autologous CAR-T cell therapies, for instance, are incredibly powerful, but they are personalized medicines, and that brings major challenges for manufacturing, scalability, and cost.

As a result, I am seeing a shift toward more platform-based thinking. Companies are looking for delivery technologies and manufacturing strategies that will support scalability, broader patient access, and more off-the-shelf solutions. It is about taking all those hard-won successes and rethinking how we can bring them to patients faster, less expensively, and with fewer infrastructure challenges.

Q What will it take for non-viral delivery systems to achieve the maturity and confidence that viral systems have reached?

BK-C Viruses and viral vectors have a natural head start because evolution has already designed them to target certain cell types, enter those cells, traffic their genetic information to specific compartments, and sometimes even permanently insert that genetic material into the host chromatin.

Take HIV, for example, which forms the basis for lentiviral vectors. You do not need to do much alteration to the vector system, because it is already designed to enter the nucleus and permanently integrate into the genome for long-term expression.

In comparison, non-viral systems such as lipid nanoparticles (LNPs) are more like transport vehicles that lack this type of embedded biological logic. When thinking about gene delivery, getting the payload into the cell is sometimes not enough. Once it's in there, you might also need it to reach the nucleus, or even integrate, depending on your therapeutic modality. Right now, LNPs do not have the 'instructions' to make that happen.

There is a lot of active work underway to determine what sorts of bells and whistles can be added to LNP systems to get cargo to the right place and express for the right duration of time. Those are the benchmarks we need to reach to bring LNPs to a comparable level of maturity to viral systems.

Q How do the analytical and manufacturing challenges differ between viral and non-viral delivery systems, and where are the biggest knowledge gaps still to be addressed?

BK-C Viral systems are still manufactured using human cell lines, which creates challenges for scalability and cost. When you purify a viral vector, you also have host cell impurities to contend with, complicating both manufacturing and analytical testing.

Analytical capabilities for viral vectors are certainly more established than they were a decade ago, and regulatory expectations are evolving and becoming more clearly defined, although those expectations are also rising.

By contrast, manufacturing for LNPs does not require cells, which theoretically makes it simpler and more amenable for scale-up. The bigger challenge is that the field is still relatively new, and we do not yet fully understand what the critical quality attributes are beyond particle size and payload encapsulation. How do we design analytical strategies that predict clinical performance, not just physical characteristics?

A key question I work on during the process of establishing manufacturing and analytical strategies is how we can design a gene delivery system and test it for long-term durability of expression or targeting. What measurable product attributes will correlate with clinical outcomes? That remains a major knowledge gap we are working to close.

Q Many developers struggle to determine what's truly phase-appropriate when building analytical packages. What do you recommend for getting this balance right?

BK-C Your data package needs to be scientifically grounded but also practical enough to support your clinical timelines and future scalability, which is a hard target to hit.

From a scientific perspective, developers must ensure they are measuring attributes tied to both the mechanism of action and risk, such as off-target effects. For newer modalities where regulatory expectations are still evolving, that understanding is critical.

From a practicality standpoint, early analytical methods should be designed with the long term in mind. You do not need a commercial-ready analytical suite from day one, nor should you build one. However, too often, teams take a 'good enough to get to

"From a practicality standpoint, early analytical methods should be designed with the long term in mind."

IND' approach, only to later discover that their methods have high failure rates, cannot accommodate scale-up, or require extensive redevelopment midstream. That causes major headaches and increases costs.

Q What does a 'science-forward' regulatory package look like in 2025, and how can developers best prepare to engage with authorities on novel delivery technologies?

BK-C It is always hard to predict the future, but I would say a science-forward package is one that shows you truly understand your platform at a mechanistic level, not just how to make it, but how it works.

There is an increasing expectation for developers to be able to articulate how their delivery system enters cells, what governs expression, and what risks are associated with each step of that process, particularly for new technologies.

When I work with teams developing viral vectors, I often tell them to 'think like a virus.' Trace the payload's journey from cell entry through expression or integration and build your data package around understanding the vector attributes that drive each step. The same principle applies to non-viral systems.

You need to show structured understanding of delivery biology, not just list interesting attributes and develop tests around them. Grounding regulatory engagement in mechanism and well-reasoned scientific questions positions you to justify your innovation and risk mitigation more effectively.

Q If we fast-forward 5–10 years, what do you envision as the defining features of the next generation of gene delivery technologies?

BK-C First and foremost, they need to be off-the-shelf and scalable. 'Off-the-shelf' means a few things to me: products that are easy to stockpile because manufacturing is not the limiting factor, and that can be administered anywhere in the world, not just where specialized infrastructure exists.

Future gene delivery technologies will also be more programmable and modular. Targeting remains a major challenge, so I would like to see platforms where you can switch out components to adjust targeting or therapeutic modality easily.

Manufacturability needs to be designed in from the start; CMC should not be an afterthought. Ultimately, the success of next-generation delivery technologies will depend on how well we align performance with real-world deployability.

Q Finally, what are your key goals and priorities over the next 1–2 years?

BK-C We are at an incredibly exciting point in gene delivery. Science has unlocked enormous possibilities, but there is still a significant

implementation gap. Many teams have excellent scientific concepts and strong preclinical proof-of-concept data, but they struggle to translate those into manufacturable, scalable, and regulatory-ready programs.

Over the next few years, my priority is to help bridge that gap, and to turn innovative delivery concepts into therapies that are positioned to reach patients.

BIOGRAPHY

Brenna Kelley-Clarke, is the Founder and Principal Consultant at Brenna Consulting, a Seattle-based firm specializing in analytical strategy and CMC guidance for viral vector development in gene and cell therapy. With more than 15 years of experience across startups and large biopharma, she helps teams design phase-appropriate analytical frameworks and drive data-informed decision-making. A virologist by training, Dr Kelley-Clarke earned her PhD from Harvard University, Cambridge, MA, USA and completed postdoctoral research at the University of Washington, Seattle, WA, USA.

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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT

NON-VIRAL DELIVERY: RESEARCH, DESIGN, AND ENGINEERING

EVENT PREVIEW

Advanced Therapies Week 2026

Cell & Gene Therapy Insights 2025; 11(11), 1347–1349 · DOI: 10.18609/cgti.2025.156

As part of our ongoing coverage of major gatherings in the advanced therapeutics space, *Cell & Gene Therapy Insights* presents a preview of Advanced Therapies Week 2026, taking place February 9–12, 2026, in San Diego, CA, USA. The meeting will convene over 2,000 attendees and 250 world class speakers from across biotech, pharma, CDMOs, academia, and investment to examine the scientific, developmental, and commercial factors shaping the next decade of cell and gene therapy (CGT). Readers of *Cell & Gene Therapy Insights* are entitled to a **20% discount on delegate tickets**–just use the code **CGTI20** when registering here.

ADVANCED THERAPIES WEEK

Here, we have compiled a preview into the standout sessions you won't want to miss.

CLINICAL DEVELOPMENT & TRANSLATION

Early and proactive regulatory interaction remains essential for reducing uncertainty in first-in-human (FIH) development. In a panel entitled Regulatory engagement for early-stage developers, Lara Ionescu Silverman (Founder and Principal Consultant, LIS BioConsulting), Anna Koptina (Head of Regulatory Affairs, Elicera Therapeutics), and Samar Mohanty (President and CSO, Nanoscope Therapeutics) will examine best practices for preparing high-quality investigational drug (IND) submissions and addressing challenges related to adaptive trial designs. The discussion will highlight strategies to streamline approvals, minimize delays, and leverage agency feedback to strengthen translational planning.

The persistent challenge of achieving translational fidelity in CGT development will be explored in the fireside chat 'Bridging the gap: overcoming challenges in animal-to-human translation' featuring Peter Francis (Chief Scientific Officer and Chief Medical Officer, Ray Therapeutics) and Rakesh Awasthi (Director, Clinical Pharmacology Cell and Gene, Novartis). Speakers will evaluate approaches to optimize preclinical models, integrate early human data into dose selection, and develop frameworks that reduce safety and efficacy uncertainties when moving programs into clinical testing.

MANUFACTURING & SUPPLY

Manufacturing strategy is increasingly a decisive factor in CGT scalability and commercial viability. The fireside chat 'Autologous vs allogeneic: manufacturing models built for scale and speed', chaired by Jessica Carmen (Chief Commercial Officer, RoslinCT), brings together Cokey Nguyen (President and CEO, Atara Biotherapeutics) and James Adams (Chief Technical Officer, Tr1X) to compare autologous and allogeneic production models. Topics will include vein-to-vein logistics, automation requirements, cell bank standardization, closed-system processing, and balancing cost of goods with operational efficiency to meet clinical demand.

For emerging biotechs, establishing strong foundations in tech transfer and manufacturing readiness is essential for avoiding costly bottlenecks during scale up. In the panel entitled, 'Tech transfer and manufacturing readiness for scaling biotech' speakers will examine how early-stage decisions shape scalability, product quality, and regulatory alignment, with insights from Poh Yeh-Chuin (Head of Technical Ops, Tolerance Bio) and Justin Skoble (Vice President of Technical Operations, Caribou Biosciences). The session will highlight how technical operations can be aligned with long-term growth strategies to support a scalable and sustainable manufacturing platform.

EMERGING MODALITIES & INNOVATION

Developing CGTs for solid tumors requires modalities capable of navigating tumor heterogeneity, immune evasion, and the tumor microenvironment. In the session 'Choosing the right modality to overcome the solid tumor challenge', Richard Koya (Professor, University of Chicago School of Medicine) will outline how modality selection influences targeting strategies, penetration into solid tumor sites, and dose and safety considerations. The session will also highlight biomarker-driven patient selection, translational considerations, and challenges in preclinical modeling that affect clinical predictability.

GROWTH & INVESTMENT

As more CGT products progress toward approval and market launch, companies face increasing pressure to establish sustainable commercial foundations. The panel 'Building a viable commercial model in CGT' featuring

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Ahmed Mousa (General Manager, Lafana Life Sciences), Anna Catalanotto (Senior Manager of Commercial Development, Cardinal HealthTM Advanced Therapy Solutions), Craig Martin (CEO, Orphan Therapeutics), Jacob Smith (Head of Tech Development and CMC, Viralgen), and Audrey Greenberg (Mayo Venture Partner, Mayo Clinic) will explore pricing and reimbursement models, payer alignment strategies, distribution and supply chain considerations, and risk-sharing frameworks that support affordability, access, and long-term value generation in the CGT sector.

Advanced Therapies Week 2026 promises to deliver one of the most comprehensive snapshots yet of the scientific, regulatory, and commercial realities shaping next-generation cell and gene therapies. From translational strategy to manufacturing scale-up, and regulatory clarity to commercial insights, the meeting will offer attendees critical insights into accelerating development and strengthening future CGT ecosystems. Together, these themes point toward a maturing field that is sharpening its focus on translational robustness, scalable manufacturing, and commercial viability.

Register here now and use the code **CGTI20** to receive a **20% discount** on delegate tickets.

Additionally, to find out what other cell and gene therapy events are upcoming, you can find our online Events Calendar **here**.

CELL & GENE THERAPY INSIGHTS

AI AND DIGITIZATION

Digitizing CMC: opportunities and constraints for artificial intelligence adoption across CGT development and manufacturing

INTERVIEW

"[...]I expect AI to accelerate readiness for clinical and commercial operations by several years, alleviating long-standing bottlenecks across development and launch."

Ashling Cannon (Commissioning Editor, *Cell & Gene Therapy Insights*) speaks with Kumar Dhanasekharan (SVP, Head of Technical Operations, Voyager Therapeutics Inc.) about the digitization barriers limiting artificial intelligence adoption in CMC and supply chain operations for cell and gene therapies, and the technical and regulatory considerations required to achieve real-time orchestration and operational resilience.

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"End-to-end integration is not new.
More than ten years ago, during my time at Genzyme,
it was already a major challenge across biotech.
Fundamentally, we lack a true plug-and-play system that
connects data and knowledge across all these platforms."

Q Given your experience overseeing CMC and supply chain operations, what do you see as the most critical digitization gaps that must be addressed before AI can reliably forecast raw material availability and orchestrate capacity across internal and CDMO networks?

KD The biggest gap across the industry today is that data and knowledge remain disconnected across sites, groups, and functions. Process development may use an electronic laboratory notebook, analytical and QC teams use a laboratory information management system, and manufacturing uses a manufacturing execution system. These systems all generate different types of data, including discrete and time-series data with different frequencies and missing points.

In addition, there are PowerPoints, spreadsheets, protocols, and reports stored across various document management systems. All of this remains fragmented, and there is no overarching way to bring these streams together. Without a unifying integration layer, AI cannot assemble a complete, contextualized view of operations.

There is also the added complexity that some data reside at CDMOs, where confidentiality limits access. Without comprehensive access, it is extremely difficult to produce reliable forecasts or orchestrate capacity and raw materials across networks.

Q End-to-end digital integration is increasingly important in CGT operations. Where are the biggest opportunities and challenges when building a digital backbone that enables real-time visibility across QC timelines and multiple manufacturing sites?

KD End-to-end integration is not new. More than ten years ago, during my time at Genzyme, it was already a major challenge across biotech. Fundamentally, we lack a true plug-and-play system that connects data and knowledge across all these platforms.

Companies have historically tried to solve this through large enterprise-level initiatives or custom knowledge management systems. These efforts are extremely expensive, and very few organizations have succeeded completely. Some of the largest companies may be closer, but the gap persists.

For CGT specifically, the need becomes critical at commercial stage when uninterrupted supply is required. In early development, patient numbers and clinical sites are small, so the pressure is lower. But across biotech, full end-to-end integration remains the ultimate ambition, and we are not there yet.

Q How far can AI-driven scenario modeling and dynamic risk triage go in improving launch readiness and operational resilience for the CGT supply chain, and what barriers still exist at the data and interoperability levels?

KD This is an area where AI can already add value. Scenario modeling and risk triage are relatively low-hanging fruit and can help teams make faster, better-informed decisions. Digital twins for equipment monitoring and early performance flagging are already in use and can support operational resilience.

The limitations appear when we try to rely on more integrated datasets. The lack of high-quality, structured, end-to-end data remains a major constraint. Even so, AI can incorporate external factors such as weather, transportation delays, and shipping conditions to reduce deviations, excursions, and product losses. For CGT products with extremely high value, this is significant.

Progress is being made across the industry, but comprehensive system learning remains restricted by data fragmentation and limited interoperability.

Q Emerging generative AI and digital copilot systems are gaining traction across the industry. Where do you see the most credible GMP-aligned use cases for CGT today, and what validation or data-quality barriers must be addressed before adoption at scale?

KD There are clear opportunities for large language models to support documentation-heavy activities. Summarizing batch records, reviewing GMP documents, and accelerating tech transfers are areas where these tools can make a real difference. If we feed the right data into these systems, they could even help generate the first draft of a regulatory submission like an IND by assembling information from multiple CMC sources.

CMC is highly documentation-driven, and writing an IND may require reviewing 100–200 source documents. Automating the first-pass drafting based on validated source materials would be a significant benefit for both regulatory and technical teams.

We are already seeing early signs of this in practice. I recently came across a material workflow that historically took several hours and had to be repeated hundreds of times each year. With AI, that same process can now be completed in just a few minutes. Additional impactful areas include documentation and tech transfer, reflecting the immediate opportunity to reduce manual review burden and accelerate development timelines. These early examples show that automation in this space is both feasible and impactful.

The key barriers are centered on validation and trust. As with any software used in regulated environments, robust verification and validation frameworks are required to ensure

"Progress is being made across the industry, but comprehensive system learning remains restricted by data fragmentation and limited interoperability."

accuracy, data integrity, and auditability. Given that AI outputs are not entirely deterministic, a human-in-the-loop can be incorporated into the validation workflow to mitigate these limitations which also supports regulatory alignment.

Q What will an AI-enabled and fully digitalized CGT supply chain look like in five years, and which capabilities do you think are technically achievable within that timeframe?

KD AI is advancing rapidly and will be disruptive across the entire development lifecycle. For CMC specifically, one of the biggest bottlenecks is tech transfer. Today, moving a process to a CDMO and generating batch records, automation recipes, and documentation typically takes six months or more. With AI-enabled documentation assembly and automation, that could potentially save several months.

IND Module 3 assembly can also take several months. AI-driven drafting, based on structured data, could greatly compress this timeline. In CGT, where supply chains involve plasmids, qualified raw materials, multiple manufacturing sites, and multiple testing labs, AI can help streamline workflows and reduce delays.

I also predict significant progress in real-time risk management, predictive QC, and dynamic scheduling within the next five years, if not sooner. CMC is often the critical path in accelerating drug development, but with AI-driven accelerated workflows, I anticipate this will no longer be the case. Regulatory acceptance will evolve in parallel, provided appropriate validation frameworks are established.

Overall, I expect AI to accelerate readiness for clinical and commercial operations by several years, alleviating long-standing bottlenecks across development and launch.

BIOGRAPHY

Kumar Dhanasekharan is Senior Vice President of Technical Operations at Voyager Therapeutics, overseeing multi-modality CMC development, manufacturing, quality control, and supply chain for AAV gene therapy and non-viral platforms. He has extensive experience building internal manufacturing capabilities and managing CDMO partnerships, advancing multiple protein therapeutics and AAV programs into clinical stages. He has led process and analytical development, Phase I/II manufacturing, late-stage characterization, validation, and BLA-enabling activities. His previous roles include SwanBio, Amicus, Genzyme-Sanofi, and Catalent. Dr Dhanasekharan holds a PhD from Rutgers University and a chemical engineering degree from IIT Chennai and has authored numerous presentations and publications.

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CELL & GENE THERAPY INSIGHTS

ANALYTICS

Fit-for-purpose analytical considerations to support the clinical readiness of engineered AAV vectors

Jorge Santiago-Ortiz and Andrew Steinsapir

VIEWPOINT

"The development of optimized analytical workflows compatible with both natural serotypes and engineered capsids ensures robust characterization of AAV vectors across different programs."

Engineered AAV capsids are designed to improve tropism, enhance immune evasion, and increase manufacturability, yet these modifications can introduce unique analytical challenges that, if unaddressed, may limit the safe and efficacious use of engineered capsids in AAV gene therapies. Compared to natural AAV serotypes, engineered capsids may exhibit altered physicochemical properties, including reduced thermostability and changes in binding affinity to antibodies used for purification or detection. These differences necessitate the development of fit-for-purpose analytical methods to ensure accurate quantification and

characterization of key quality attributes for AAV gene therapies. Here, we outline which commonly used assays are expected to be robust across capsids and which may require adaptation, and propose a fit-for-purpose, orthogonal analytical strategy to reliably characterize engineered AAV vectors, a critical step towards their safe and efficacious use in gene therapy products.

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INTRODUCTION

AAV vectors are composed of a protein capsid that packages a single-stranded DNA genome; the mature AAV capsid consists of a total of 60 monomers of three distinct structural viral proteins (VPs) encoded by a given capsid's *cap* gene, known as VP1, VP2, and VP3, which assemble to form an icosahedral shell [1]. The amino-acid sequence of these proteins determines a capsid's structure and its physicochemical and biological properties, which in turn influence key gene delivery properties including receptor engagement, tropism, immunogenicity, and stability [2]. Differences in properties can be observed across AAV natural serotypes as a result of their distinct amino-acid sequences [3]. Engineered AAV capsids, which may incorporate modifications such as peptide insertions, site-specific mutations, chimeric sequences, or chemical conjugations [4], may differ sufficiently in their properties to necessitate capsid-specific, fit-for-purpose analytical method development. Establishing such methods is essential to ensure that engineered capsids can be appropriately characterized and supported for safe and efficacious use in gene therapy products.

ANALYTICAL CONSIDERATIONS FOR ENGINEERED AAV CAPSIDS

Table 1 presents a list of quality attributes that are commonly examined as part of the analytical release of AAV vector preparations [5], as well as examples of corresponding analytical assays. Each analytical method relies on specific physicochemical or biological properties intrinsic to each AAV vector. Certain properties are expected to vary only modestly across individual capsids (i.e., capsids that are not conjugated to other molecules); these include total capsid protein content, capsid molecular mass, vector particle density as a function of packaged genome, and hydrodynamic size. Consequently, corresponding analytical methods such as bicinchoninic acid assay, Bradford assay, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), mass photometry, cryogenic transmission electron microscopy, and dynamic light scattering are expected to require minimal (if any) refinements to become suitable for the analysis of natural AAV serotypes and engineered capsids alike, provided sample preparation conditions (e.g., buffer, surfactant, concentration) are appropriate for the capsid in question.

Other properties may vary more significantly when working with engineered AAV capsids. Capsid modifications can impact solution stability, leading to increased material losses while handling or diluting virus material when buffer conditions are suboptimal [6]. This underscores the importance of using a suitable virus formulation buffer that maintains an appropriate pH and contains suitable salts and surfactant as a diluent during all dilution and sample preparation stages for analytical testing.

Due to their modifications, engineered capsids can have lower thermostability compared to natural AAV serotypes or their parental capsid [7]. As a result, engineered capsids may be incompatible with

TABLE 1

Capsid-specific quality attributes and associated assays requiring fit-for-purpose method-suitability confirmation to support clinical readiness.

Attribute category	Quality attribute	Assay	Key analyte properties	Recommendation
Strength	Total protein concentration	BCA, Bradford, UV280	Presence of amino acid residues	No expected capsid-dependent issues; confirm suitability under routine conditions
Strength	Genomic titer (viral genomes per unit volume)	ddPCR, qPCR	Thermostability	Confirm suitability of genome extraction step; chemically inhibit nuclease prior to heating
Strength	Potency	Cell-based transduction assay	Transduction efficiency	Confirm transduction of cell line; adjust MOIs if needed
Strength	Capsid titer (capsids per unit volume)	Capsid ELISA	Protein sequence; binding affinity to ELISA ligand	Confirm binding to commercially available reagents; confirm suitability of available empty vectors as reference material
Strength	Infectious particle titer (TCID50)	Transduction of permissive cell line + genomic titer assay	Transduction efficiency; thermostability (genomic titer assay)	Confirm transduction of cell line; adjust MOIs if needed; follow recommendations for genomic titer assay
Safety	Presence of replication competent AAV	Transduction of permissive cell line + genomic titer assay	Transduction efficiency; thermostability (genomic titer assay)	Confirm transduction of cell line; adjust MOIs if needed; follow recommendations for genomic titer assay
Purity	General purity	SDS-PAGE	Presence of amino acid residues; protein size	No expected capsid-dependent issues; confirm suitability under routine conditions
Purity	Full/empty capsid ratio	Mass photometry	Particle molecular mass	No expected capsid-dependent issues; confirm suitability under routine conditions
Purity	Full/empty capsid ratio	Cryo-Transmission Electron Microscopy (cryoTEM)	Particle density	No expected capsid-dependent issues; confirm suitability under routine conditions
Purity	Full/empty capsid ratio	Genomic titer + capsid ELISA	Particle density	Follow recommendations for genomic titer assay and capsid ELISA assay
Purity	Aggregation	Dynamic light scattering	Hydrodynamic size distribution	No expected capsid-dependent issues; confirm suitability under routine conditions
Identity	Capsid protein identity	Western blot	Binding affinity to detection antibody	Confirm binding to commercially available antibody; confirm suitability of routine conditions for appropriate detection
Identity	Genomic identity	Sequencing	Thermostability (viral genome extraction step)	Confirm suitability of genome extraction step; chemically inhibit nuclease prior to heating
Identity	Genome integrity	Deep sequencing methods; CE-LIF	Thermostability (viral genome extraction step)	Confirm suitability of genome extraction step; chemically inhibit nuclease prior to heating

certain analytical method processing steps involving the heating of a vector sample above 37°C, as is done in viral genome extraction methods employed for genomic titer determination, genome sequencing, and genome integrity analysis. For example, some viral genome extraction methods in genomic titer assays heat-inactivate DNase I before capsid digestion/denaturing: they may proceed from a 37°C incubation step to a 65+°C enzyme inactivation step before continuing with capsid digestion/denaturing. It is critical that DNase I (or the alternate nuclease being employed) be fully inactivated before the sample is heated above 37 °C. One practical approach is to add sufficient ethylenediaminetetraacetic acid to fully chelate the divalent cations required for nuclease activity before the heating step. Otherwise, residual enzyme activity can digest prematurely released viral genomes during the heating step, leading to an under-counting of viral titer [6,7]. This may happen regardless of whether a proteinase-based method or a heat-based method is being used for viral genome release. Consequently, viral genome extraction steps may need to be adapted in assays for genomic titer, genome sequencing, and genome integrity.

Surface-exposed modifications on engineered capsids may reduce or ablate binding to antibodies and other affinity reagents; this can impact downstream processing (when using affinity resin for capture chromatography) as well as Western blot and capsid ELISA assays. Routinely used anti-AAV Western blot antibodies or capsid ELISA kits may not be compatible with engineered capsids despite working with their parental capsid [6], which may motivate the development of assays using alternative antibodies or affinity ligands with binding epitopes that are preserved in the engineered capsid, respectively.

Engineered capsids may exhibit altered transduction properties, which are often improved under conditions where their target receptor is sufficiently expressed, including cells cultured *in vitro*. This may enable more facile development of potency assays with lower multiplicities of infection compared to the parental capsid. Accordingly, cell-based potency assays, infectious particle titer assays, and replication-competent AAV assays may require MOI and readout adjustments for each engineered capsid relative to its parental serotype.

RECOMMENDATIONS

Confirming the suitability of analytical methods to characterize different AAV vectors is integral to the development of manufacturing processes and the advancement of gene therapy programs. Such analytical readiness is critical for identifying capsid-specific risks and ensuring the safe and efficacious use of engineered capsids in gene therapy products. When evaluating a new engineered capsid, existing qualified assays cannot be assumed to be directly transferable; instead, assessments are required to determine whether each method remains appropriate given the capsid's physicochemical properties. A structured method qualification extension process, guided by the specific features that differ from the parental or historical capsids, can be undertaken to confirm assay suitability. An effective first step is to assess how the engineered capsid differs in sequence, structure, and predicted physicochemical properties. These differences can then guide suitability assessments across analytical methods.

Generating a reference standard for the target AAV capsid can further support analytical development and the confirmation of the suitability of each individual analytical method. Such a standard can ideally be produced using manufacturing processes and qualified analytical assays that have been confirmed to be compatible with other engineered capsids that have been generated using similar modification

strategies. In the absence of such a standard, AAV vector preparations should be carefully analyzed by combining assays whose readouts may differ across capsids with orthogonal methods expected to behave consistently across capsids. For example, complementing genomic titer data with results from bicinchoninic acid assay, SDS-PAGE, and mass photometry assays can provide a more complete analysis of the virus material and facilitate comparisons to historical data from the manufacturing of other capsids.

CONCLUSIONS

The development of optimized analytical workflows compatible with both natural serotypes and engineered capsids ensures robust characterization of AAV vectors across different programs. This is important for therapeutic developers, academic vector cores, and CDMOs, which routinely produce a diversity of AAV capsids. Analytical methods traditionally used for characterizing natural AAV serotypes must be evaluated to confirm their suitability and applicability for analyzing engineered AAV capsids. Evaluating a new engineered capsid may therefore require reassessing analytical methods in the context of the capsid's specific physicochemical properties rather than assuming direct assay transferability. The capsid-specific quality attributes summarized in Table 1 can further support a structured, risk-based assessment of which assays warrant the most immediate investigation, consistent with Quality-by-Design principles [8]. Methods that rely on properties that vary minimally as a result of engineered capsids' modifications, such as SDS-PAGE or mass photometry, may often be employed across capsid types after verifying that sample preparation and assay conditions are appropriate for the specific capsid. Other methods, like genomic titer and capsid ELISA assays, may require refinements to address engineered capsids' physicochemical properties. These refinements enhance the reliability of AAV drug product characterization, supporting clinical translation and manufacturability.

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CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Optimizing upstream cell culture with rapid at-line and in-line analytics

Nick Randall and Ethan Bossange

Cell therapy manufacturing faces significant variability in materials and processes, but the use of advanced analytical tools can help identify and control these sources of variation—improving cell culture consistency, increasing yield, and ensuring reliable product quality. This article discusses the use of process analytical technology (PAT) tools to address these challenges by enabling real-time monitoring of critical process parameters including amino acids, glucose, lactate, and biomass. Additionally, case studies from Ultragenyx, Emory University, and Resilience demonstrate how these technologies streamline analytics, enhance process understanding, improve productivity, and reduce costs.

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ADDRESSING CHALLENGES IN CELL THERAPY MANUFACTURING WITH REAL-TIME PROCESS MONITORING

Cell therapy manufacturing presents multiple challenges, including variability that can arise from materials (e.g., from cellular matrix cell viability, patient/donor status, media, growth factors, and serum), or the process itself (e.g., from expansion vessels, culture parameters, operators, and off-line testing). In order to improve process outcomes, these sources of variation must be better characterized and controlled by leveraging real-time monitoring and analytics.

Another significant challenge in the development of advanced therapeutics is cost. A 2020 study examined drug development sites and analyzed the length and expense of process development, clinical trials and manufacturing. It found that these costs exceed $80 million over a 5-year period which equates to approximately $1 million per month [1].

Beyond cost, time is also a significant concern—analytical delays often contribute to extended timelines in process development. In a typical workflow, several weeks are dedicated to running a bioreactor. Samples are then carefully collected and sent to analytical teams, either in-house or externally. Once the results are received, several additional weeks may be spent debriefing and planning the next run.

To address these challenges and enable process controls in a benchtop bioreactor scale, advanced tools such as PAT can be deployed. They enable at-line and in-line real-time monitoring of key process parameters, allowing different aspects of the process to be controlled as the scale is increased, rather than being limited to solitary or smaller-scale vessels. Additionally, PAT tools can significantly reduce timelines, as complex analytics are performed in-house and results are obtained within minutes, approaching real-time feedback.

Traditionally, most processes are carried out in small-scale vessels, and under infrequent off-line monitoring conditions, which often leads to high variability of cell culture health, viability, and expansion (Figure 1A). In contrast, when a bioreactor with real-time monitoring and control is used, higher consistency, yields, and cell expansion can be achieved (Figure 1B).

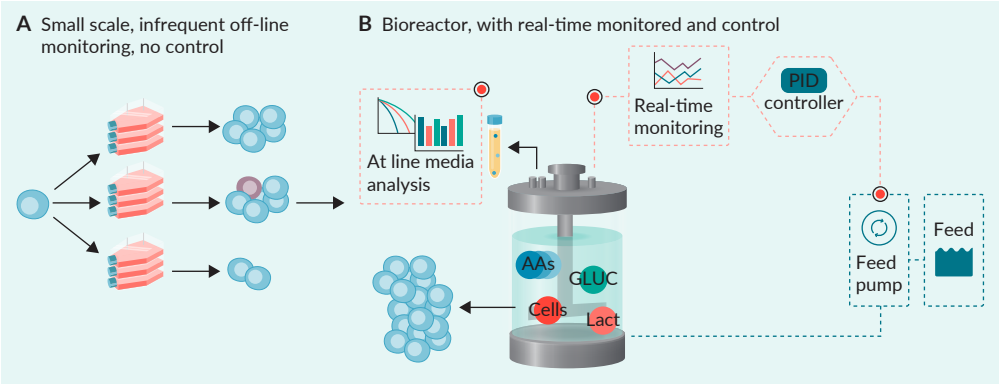
For example, PAT-based platforms, such as the REBEL® XT at-line media analyzer, can be utilized to provide immediate feedback on amino acid consumption or accumulation. Additionally, MAVEN® technology can be utilized for online glucose and lactate monitoring, while MAVERICK®, a Raman-based technology, can be used for in-line monitoring of multiple critical process parameters (CPPs). Furthermore, downstream processing technologies, such as SoloVPE® PLUS and FlowVPX®, are designed for at-line or in-line concentration monitoring and can be integrated into real-time process management systems to enable comprehensive process control.

ENHANCING PROCESS DEVELOPMENT THROUGH REAL-TIME AMINO ACID ANALYSIS

In bioprocessing, it is crucial to measure amino acid concentrations, considering these molecules act as building blocks, energy sources, and are key components in cell metabolism. In cell therapy manufacturing, amino acids are known to be complex to manage, presenting both challenges and opportunities. For instance, if concentrations are too low, it may lead to compromised process robustness, reduced growth rates, limited protein production, negatively affecting the protein sequence and overall product quality. Conversely, if concentrations of certain amino acids are too high, culture overgrowth can occur, and undesired product quality variants, such as charge variants, can be produced,

FIGURE 1

Comparison between small-scale off-line monitoring and a bioreactor with real-time monitoring and control in cell therapy manufacturing.



(A) Small-scale off-line monitoring and (B) a bioreactor with real-time monitoring.

ultimately compromising process robustness. Therefore, maintaining a balanced composition of amino acids and other components is essential for sustaining a healthy culture.

Advanced PAT-based tools, such as REBEL XT, can be used to measure amino acid concentrations in real-time. The analysis is label-free, supporting high-throughput media analysis while requiring only approximately 10 µL of sample from the bioreactor, which is particularly advantageous for cell and gene therapy (CGT) applications where limited material is available. Each run simultaneously examines over 30 standard cell media and bioprocess nutrients as well as metabolites, at significant savings compared to third-party testing.

The REBEL XT platform also supports high-throughput operation, enabling the analysis of 96-well plates or individual samples, thereby providing flexibility for various experimental setups. The system is designed to provide fast, at-line intelligence, with each sample requiring approximately 15 minutes, depending on the number of replicates or standards analysed. Overall, advanced analytical tools such as REBEL XT can enhance efficiency and cost-effectiveness in process development by reducing timelines and contributing to a lower cost of goods.

Overview of the REBEL XT components and workflow

The internal components of the REBEL XT system can be grouped into four primary categories. The first is the autosampler, which is designed to deliver samples from 96-well plates or chromatography vials, diluting them appropriately for analysis by the device.

The second component is the separation technology, which employs capillary zone electrophoresis. This technology enables analytes to migrate through a glass channel based on their size and charge before being electro-sprayed from the tip of the chip into the microscale mass spectrometer.

The third component is identification using microscale mass spectrometry to detect and characterize each analyte.

Lastly, the fourth component is the data analytics functionality, which automates analyte identification, quantifies each analyte, and generates comprehensive reports.

The REBEL XT workflow begins by collecting and processing the samples in the usual manner, including centrifugation and filtration. A diluent is then added to adjust amino acid concentrations to the appropriate levels. Samples are then loaded into the instrument, and the system is informed of sample placement. Once the process is initiated, results are obtained within minutes. The analyte panel consists primarily of amino acids along with a few biogenic amines.

Case study 1: optimizing AAV production for gene therapy using REBEL XT

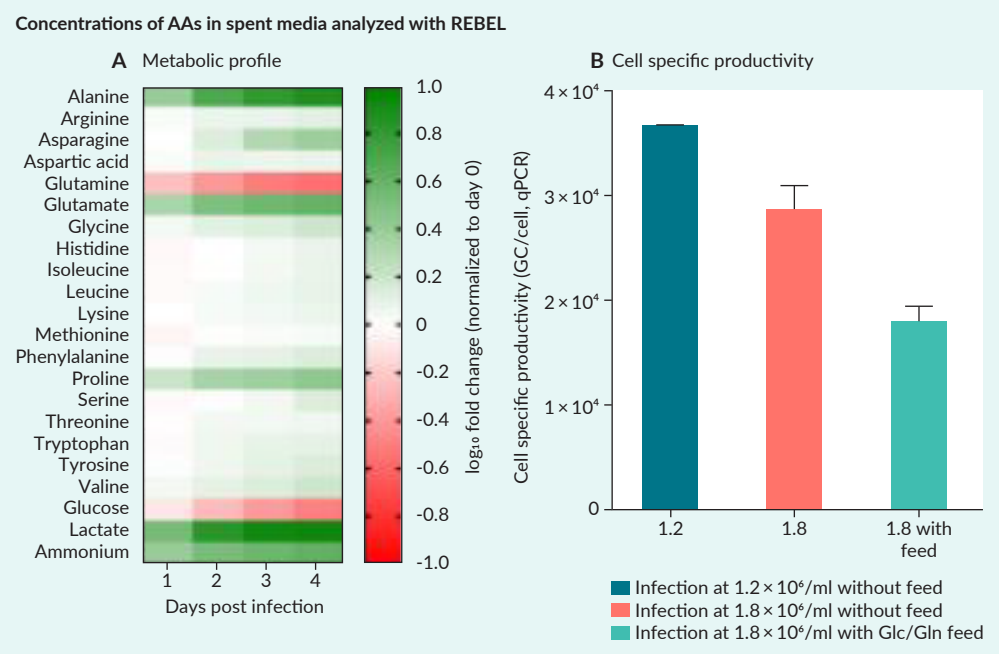
Based on a study by a group at Ultragenyx, which leveraged an AAV perfusion process, REBEL XT data can be used to optimize AAV titers in a stable cell line for gene therapy manufacturing. The goal of the study was to determine whether nutrient and amino acid deficiency could be responsible for low specific productivity and rAAV titer in the producer cell line.

The initial hypothesis was that REBEL XT could be used to identify deficiencies or amino acids that were being consumed too frequently, and that this information could be applied to enhance cell-specific productivity. In the experiment, infection times were varied, with and without feeding, and daily amino acid levels were measured. The objective was to evaluate which feed levels had the most significant impact, on optimizing conditions for increased titer.

In one experiment (Figure 2A), it was discovered that only glucose and glutamine

FIGURE 2

Concentrations of amino acids in spent media analyzed with REBEL XT.



(A) Metabolic profile. (B) Cell-specific productivity.

levels were depleted during culture, and feeding with these metabolites did not improve cell-specific productivity (Figure 2B), leading to the conclusion that nutrient deficiency is not the cause of the cell density effect in the producer cell line platform. Interestingly, it was observed that feeding with glutamine specifically decreased overall titer, illustrating that excessive supplementation can negatively affect the process.

Although the effect is cell line- and modality-specific, in the case of Ultragenyx, using feed without glutamine led to an improved overall titer without adversely affecting cell density.

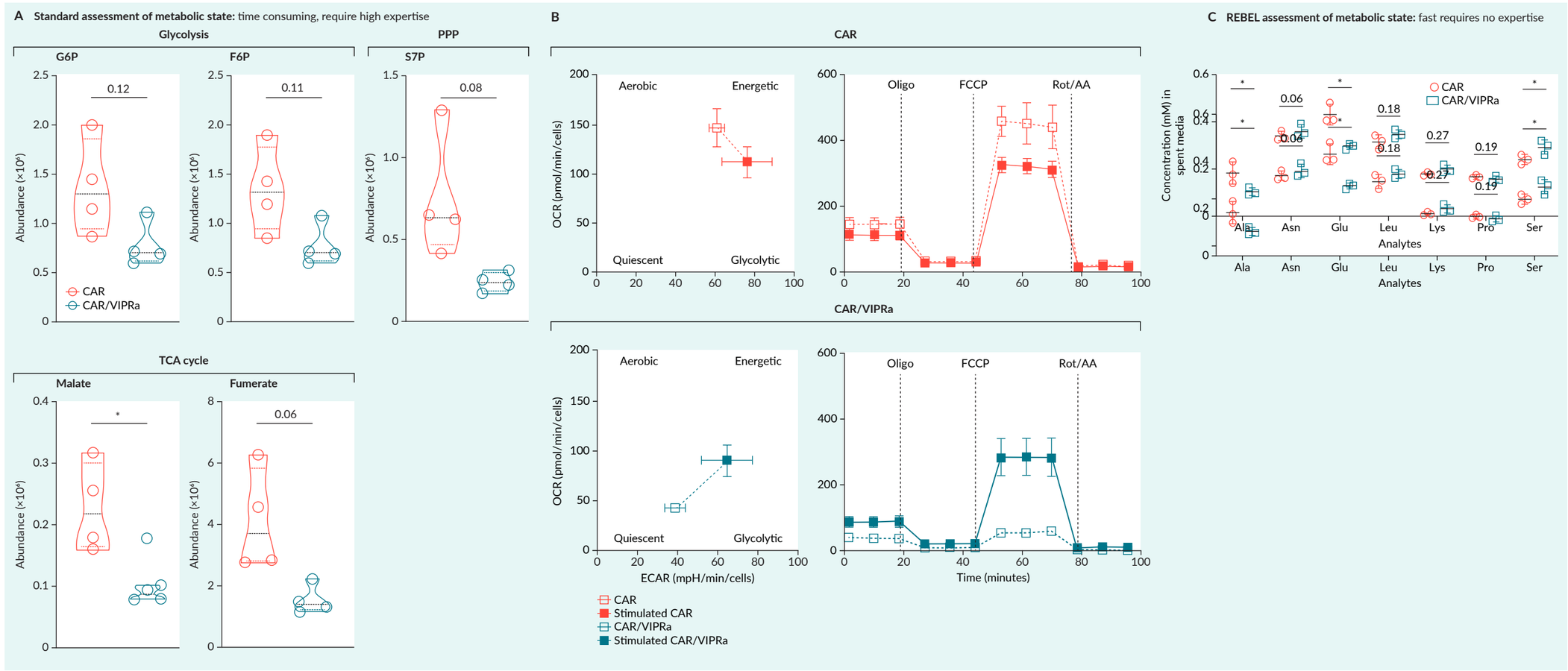
Case study 2: optimizing CAR-T cell analytics with REBEL XT

Another case study involves a cell therapy manufacturing example from the Rafiq Laboratory at Emory University. The goal of the study was to optimize the process for analysing the metabolism of their specific cell therapies and to understand how these metabolic profiles impact overall efficacy. The REBEL XT-based workflow and results were compared to standard methodologies used for assessing metabolic states.

The traditional liquid chromatography-mass spectrometry method (Figure 3A) requires hours for sample preparation, data acquisition, and analysis before results are obtained, and it is highly dependent on specialized expertise. Similarly, the results from the Seahorse mitochondrial stress test (Figure 3B) demand significant time, effort, expertise, and sample volume, and these factors can be limiting in cell therapy applications. The results obtained using the REBEL XT at-line amino acid analysis are displayed in Figure 3C. This workflow requires minimal sample volumes (10 µL) and reports results in 15 minutes, with no data processing required by the user. Furthermore, data analysis is straightforward, leveraging outputs from REBEL XT

FIGURE 3

Comparison of three methods for CAR-T cell therapy analytics.



(A) Liquid chromatography-mass spectrometry. (B) Seahorse mitochondrial stress test. (C) REBEL XT.

and software such as JMP, with no specialized expertise required. This dataset demonstrates how productivity is linked to the metabolic phenotypes of specific CAR-T cells and how REBEL data is used to generate actionable insights.

This study demonstrates the ability to correlate CAR-T cell therapy products with desired metabolic phenotypic profiles by REBEL XT measurements, enabling easy access to actionable information for process development.

Case study 3: Improving process development through REBEL XT-informed feeding strategies

At Resilience, a similar workflow was implemented, integrating REBEL XT into the standard process development platform. The objective was to better inform process development, particularly in terms of feeding strategies. This approach provided improved insights into cellular behaviour and metabolic activity, which enabled optimization of several processes, including a monoclonal antibody perfusion process.

The workflow involved sample collection, analysis using REBEL XT, and evaluation of amino acid depletion in spent media. By comparing the concentrations in fresh and spent media, consumption rates were determined, and select nutrients were replenished. This approach resulted in a 50% increase in titer, exceeding the initial target of approximately 10% [2]. Product quality was also improved, and the overall cost of goods manufactured was reduced. The process was completed rapidly, without extending timelines, and the number of planned experiments for this product was reduced.

Overall, this study demonstrates how REBEL XT data can be utilized to construct metabolic profiles and assess the effects of media composition and media exchange on differentiation.

REAL-TIME GLUCOSE MONITORING & AUTOMATED FEEDING TO IMPROVE PROCESS PERFORMANCE

Beyond amino acids, it is also crucial to measure glucose levels during CGT manufacturing. Based on data published in 2015, glucose and lactate have the highest impact on cell culture parameters, including viable cell density, titer, and viability [3]. Glucose enables the management of harmful inhibitory components, such as lactate, ammonia, and osmolality, which subsequently have downstream effects on product quality. Therefore, continuous control of glucose leads to optimal cell culture conditions and critical quality attributes (CQAs), which can significantly improve product production performance.

The standard glucose monitoring workflow currently involves offline monitoring with manual bolus feeding. This approach results in relatively large fluctuations in cell metabolism, which increases the concentration of inhibitory components, leading to the accumulation of toxic metabolites, suboptimal product quality, and reduced production rates.

Improvements can be achieved by implementing PAT-based real-time monitoring with automated bolus feeding. This reduces fluctuations, lowers toxic metabolite concentrations, improves response times, and enhances growth profiles and productivity. The process can be further optimized through real-time monitoring combined with continuous automated feeding. This approach enhances process robustness, reduces operator-related errors, and ultimately enables tighter control and improved product quality.

In addition to the technical advantages, significant cost benefits are associated with implementing PAT-based feedback control. While improvements in productivity and product quality are primary objectives, cost savings are also substantial. A 2020 study conducted by the BioForum group, which included outreach to several publications and customers in the biopharmaceutical space, attempted to quantify potential cost reductions for traditional manufacturing processes [4].

Although cost savings vary widely between different processes and products, the overall conclusion is that substantial cost reductions can be achieved with PAT-based workflows. Reduced manual sampling, minimized operator intervention, lower batch contamination risk, and more precise process control all contribute to a decrease in cost of goods.

The increased costs in bioprocessing are largely driven by factors affecting process robustness, such as sterility risks and operator errors, which can compromise repeat studies and batch runs. In a typical offline or at-line workflow, samples are collected manually, introducing sterility risks and variability. The samples are then analysed, results reviewed, and feed concentrations calculated, creating multiple opportunities for error. Subsequently, the feed is prepared and added, and the process is repeated to monitor and verify, with additional adjustments made as needed.

The implementation of an in-line or online workflow addresses these challenges. Continuous measurements and automated feeding reduce the variability and risks associated with manual sampling. Additionally, dynamic feed control enables more precise management of the process, decreasing labour requirements and the cost of goods. Overall, this approach enhances process robustness, productivity, and product quality.

Real-time glucose and lactate monitoring using the MAVEN platform

PAT-based platforms, such as MAVEN, can be utilized for online monitoring of glucose and lactate. The system is

enzymatic-based and utilizes diffusion sampling, enabling highly precise measurements, even at low glucose concentrations, ranging from 0.01 g/L up to 40 g/L.

Furthermore, MAVEN enables the accurate control of substrate feeding at very low levels or at the optimal level for the metabolism of a specific cell line. Importantly, continuous monitoring and control can be achieved without removing any volume from the bioreactor.

MAVEN is compatible with a wide range of bioreactors, allowing seamless integration into closed-loop cell therapy platforms, benchtop bioreactor scales, and larger fermenters, making it highly adaptable. Finally, the system is designed for ease of operation, requiring minimal customer expertise or intervention.

The operation of MAVEN relies on the combination of diffusion technology and enzymatic biosensing. Typically, during an analysis, buffer flows from the buffer bag through the tubing set and into the sampling accessory. In this case, the sampling accessory is a stainless-steel probe integrated into the top port of a bioreactor, although in some cases, a single-use or reusable flow cell may be employed.

All configurations utilize the same underlying technology, in which a membrane is exposed to the cell culture media. This membrane has a defined cutoff, preventing larger components such as cells, the product of interest, or bacteria from migrating through. During the accumulation period, when the flow of buffer is stopped, small molecules diffuse across the membrane into the channel of the sampling accessory. Upon resumption of buffer flow, these small molecules are transported through the tubing set back to the device, where they are measured using the enzymatic biosensor.

Enzymatic biosensing is commonly used in the industry because enzymes exhibit high selectivity, binding only the analytes of interest—in this case, glucose and lactate. This interaction generates an electrochemical signal that is proportional to the amount of glucose and lactate sampled.

The resulting data are then communicated digitally, either via OPC-UA or directly to a feed pump, enabling various feedback control solutions. Measurements can be performed as frequently as every 2 minutes. This rapid monitoring is achieved through the continuous flow of fluidics through the device, sampling, and detection. The combination of diffusion sampling and enzymatic biosensing thus provides real-time data without any loss of bioreactor volume.

In-line monitoring of glucose, lactate, and biomass using the MAVERICK system

MAVERICK is a Raman-based system designed for in-line monitoring of three key process parameters: glucose, lactate, and total biomass. Raman technology is optical-based and enables the simultaneous measurement of several parameters. MAVERICK was developed to provide the benefits of Raman technology while minimizing implementation hurdles. It is designed to function similarly to a pH meter and is generalizable across various cell lines, bioreactor scales, and media.

MAVERICK features a rapid calibration and performance qualification, taking approximately 10 minutes and largely automated via the user interface. The probe would be connected to the bioreactor and then autoclaved for sterility. After reconnection, continuous data collection for glucose, lactate, and biomass begins. Extensive engineering ensures repeatability, reduces guesswork, and creates a plug-and-play system.

The system includes an immersion probe with an incorporated microchip for traceability, connected via an optical cable to the measurement module, which houses

the spectrometer. Each Measurement Module has individual analog outputs for feedback control and connects to the Hub through a single cable, providing power and communication. The Hub functions as the central control unit, running the user interface, embedded *de novo* model, reporting results, and enabling system functionality. Up to six Measurement Modules can connect to a single Hub, supporting multiplexing and advanced DOE applications.

The Hub software displays real-time results, controls start and stop functions, and supports 21 CFR Part 11 compliance. The only consumables required are the calibration standards kit, used to calibrate the device, ensure electromechanical functionality, generate pass-fail reports, and confirm model performance.

MAVERICK utilizes a *de novo* model, measuring parameters in real-time and not trained on empirical data or historical runs. The model comprises both static and dynamic factors. The static factors would include Master Formulary which captures spectral fingerprints of hundreds of media components, enabling the system to interpret how concentrations in complex mixtures affect measurement results. Dynamic components compensate for variations in the spectrometer and environmental factors, enabling fully automated operation under standard laboratory conditions.

When a measurement occurs, light is emitted by the laser and travels through the fiber optic cable to the immersed probe. The light interacts with chemical bonds, causing Raman scattering at different wavelengths. Scattered light travels through the fiber optic cable to the detector array, where spectra are recorded and sent to the Hub. Relative peak intensities indicate relative concentrations, whereas absolute peak intensities directly correlate with the concentration of individual chemical species.

In essence, this principle is consistent with conventional Raman technology; however, the novelty of MAVERICK lies in the automated processing of the spectra. The Raman spectrum is automatically saved and routed through the *de novo* model to generate real-time concentrations of glucose, lactate, and biomass. These measurements can then be used for feedback control by routing signals, either digitally or via analog, to a pump controller. Measurements are taken as frequently as every minute, with each spectrum processed automatically.

Case study 1: In-line monitoring in HEK293 cell culture with MAVERICK

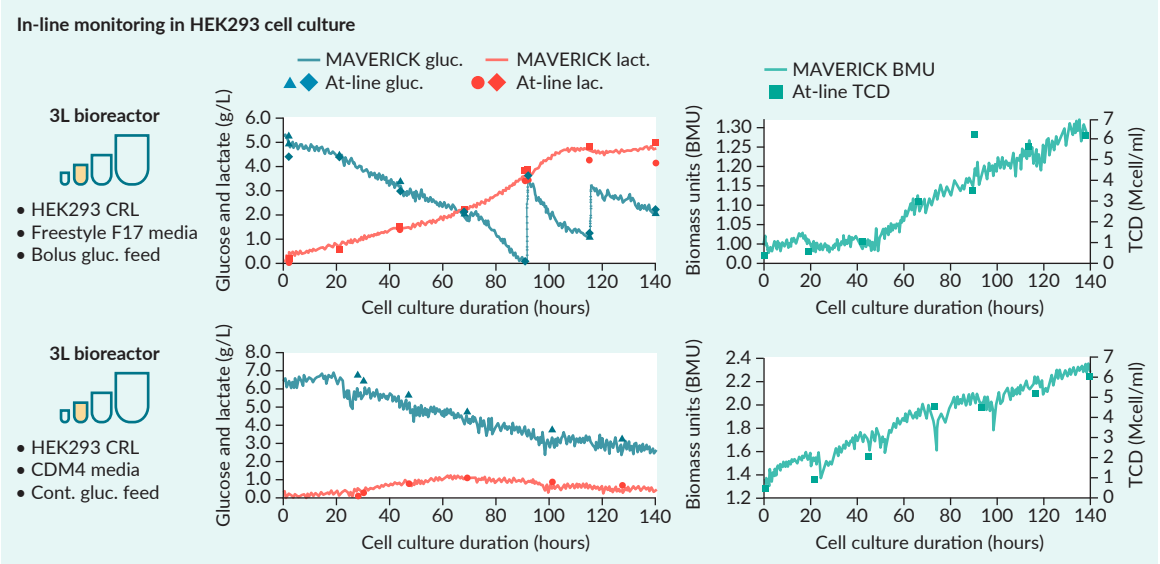
In a case study conducted in collaboration with Professor Yoon's laboratory at UMass Lowell, MAVERICK was integrated in-line during a HEK293 cell culture process. The objective was to evaluate the comparability of in-line measurements with those obtained offline using a FLEX2 and a Cedex.

For lactate, it was observed that both FLEX2 and a Cedex produced differing results, highlighting that all analytical technologies inherently carry some bias (Figures 4A and C). However, MAVERICK demonstrated strong alignment with both glucose and lactate measurements when compared to the offline systems.

Furthermore, the results illustrated in Figures 4B and D represent the correlation between biomass measurements obtained by MAVERICK and total cell density determined by an offline Vi-CELL. These experiments were performed in a 3 L bioreactor using two different types of media. Additionally, a comparison was made between bolus and continuous glucose feeding. The results are shown for both conditions, providing a clear example of how in-line measurements can closely align with offline data while offering more granular insight into what is happening between those daily time points.

FIGURE 4

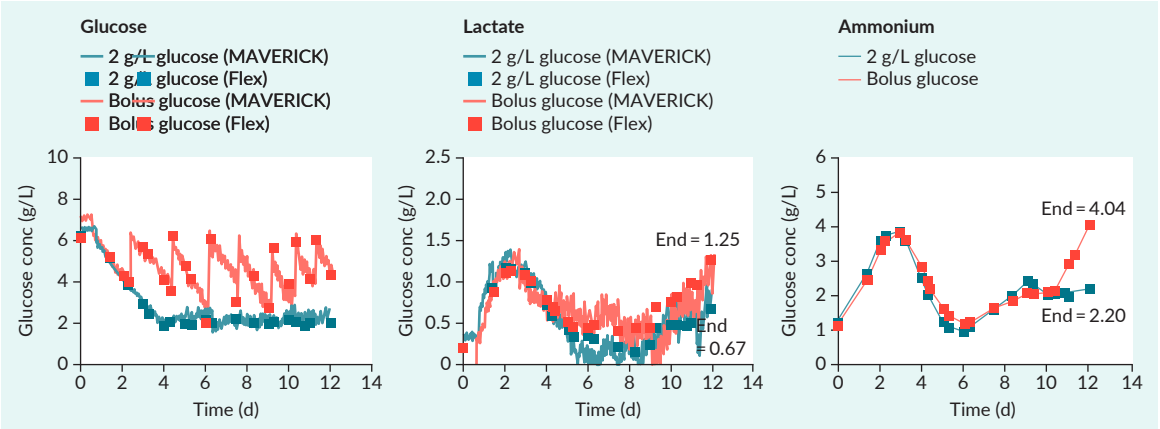
In-line monitoring in HEK293 cell culture.



A and C: the traces for glucose (in blue) and lactate (in red) are shown. The triangles and rectangles represent offline measurements, demonstrating how the in-line measurements correlated with these offline values. B and D: biomass units.

FIGURE 5

Comparison of (A) glucose, (B) lactate, and (C) ammonium levels under bolus versus continuous feeding.



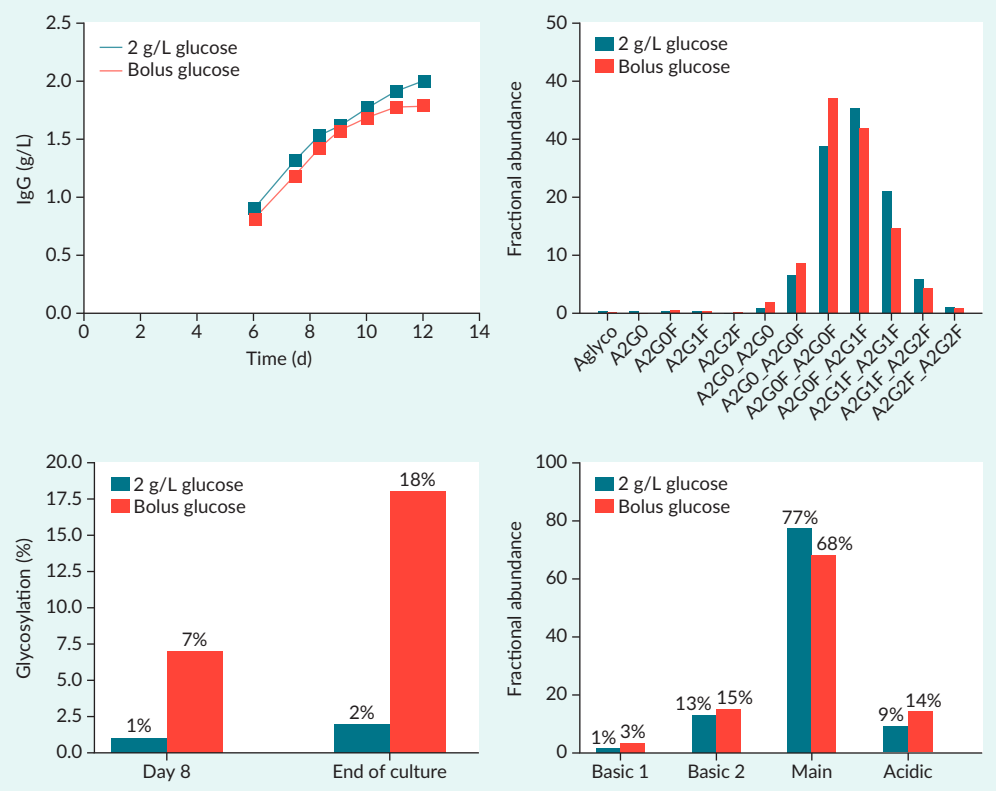
NIST-CHO cells were cultured in a 3 L bioreactor with traditional bolus feeding (4–6 g/L, orange) or continuous feeding (2 g/L, blue).

Case study 2: continuous glucose feed monitoring with MAVEN and MAVERICK

An internal case study was conducted for both MAVEN and MAVERICK to evaluate how continuous monitoring not only aligns with offline measurements but also impacts process performance and CQAs. The primary goal was to compare results between traditional bolus feeding, using a threshold of 4-6 g/L, with a continuous feeding strategy at a 2 g/L set point in the same process. The experiments

FIGURE 6

Effect of continuous glucose feeding.



(A) Titer. (B) Gycosylation profile. (C) Glycation. (D) Charge variant.

were performed using a NIST-CHO cell line in a benchtop-scale 3 L bioreactor. Offline measurements were used for reference and verification, employing both a FLEX2 and a Vi-CELL system. Several CQAs were analysed using ZipChip coupled with the Orbitrap Exploris™ 240 mass spectrometer.

Glucose, lactate, and ammonium were examined, and a strong correlation with offline measurements was observed (Figure 5). Notably, the bolus feeding produced the expected oscillating metabolic traces, whereas the 2 g/L glucose feed remained stable throughout the 12-day run. For lactate, differences became apparent toward the middle of the process. The objective was to reduce lactate accumulation toward the end of the run. At day 12, lactate levels reached 1.2 g/L with bolus feeding, compared with 0.6 g/L under continuous feeding, representing a 50% reduction. This demonstrates that continuous feeding limits unnecessary glucose accumulation, thereby preventing excessive lactate buildup. Ammonia levels were also impacted by glucose feeding. By reducing the glucose feed from 4 g/L in the bolus approach to 2 g/L in the continuous approach, ammonia levels were decreased by approximately 50% by the end of the run. This provides a clear example of how glucose control can effectively mitigate undesirable metabolite accumulation.Furthermore, the impact on process performance was evaluated. When the titer was examined (Figure 6A), it was observed that the continuous glucose feed at 2 g/L resulted in a 12% increase compared with bolus feeding.

Notably, the glycosylation profile was improved with the 2 g/L continuous

feed compared with the bolus feed (Figure 6B). Additionally, glycation and charge variance were favourably affected (Figures 6C and D). Glycation levels were reduced, resulting in a more homogeneous glycan profile, while charge variance showed a higher proportion of the main peak species and fewer variant species.

These results indicate that modulating glucose in the process not only enhances productivity and reduces toxic metabolite accumulation but also improves control over CQAs.

TRANSLATIONAL INSIGHT

Advanced analytical tools such as PAT-based systems can automate upstream workflows, reduce time to market, and provide actionable insights. They enable a clear understanding of process dynamics, allowing changes to be made in real-time rather than waiting for results from offline analyses or external analytics providers.

Platforms presented in this article, such as REBEL XT, MAVEN, and MAVERICK, enable in-line and at-line measurement of amino acids, glucose, lactate, and biomass, improving process consistency, reducing metabolite accumulation, and supporting optimized feeding strategies in bioprocess development.

This capability enables greater control over processes, ensuring maximum robustness, productivity, and product quality. Lastly, labor, time, and sterility risks can be minimized, which is particularly valuable in the CGT space.

Q&A

Nick Randall (left), **Ethan Bossange** (right)

Q How do you view the current state of PAT in CGT manufacturing, and what areas require improvement to enable wider adoption?

NR PAT is particularly beneficial in the CGT field, where cost reduction is essential to improve patient access, and the industry is gradually adopting this technology in its various forms.

On the gene therapy side, improving titer and productivity is critical to making processes more scalable and commercially viable. Currently, the field is in an early adoption phase, although adoption is somewhat more advanced in the biologics space due to the maturity of those platforms. PAT is increasingly recognized as a key method for process intensification, optimization, and understanding the dynamics of novel modalities.

EB Making PAT more accessible and easier to integrate can further facilitate adoption. For example, ensuring that flow cells are compatible with existing equipment is crucial for facilitating adoption.

Q What CPPs or attributes require more active PAT solutions for effective control?

NR Beyond more commonly monitored analytes, such as glucose, lactate, and amino acids, other small molecules in the extracellular environment, such as ammonia, are also critical. The importance of specific analytes varies depending on the modality used by customers.

One trend across the industry, particularly in cell therapy, involves analytes such as cytokines or the development of innovative technologies in flow cytometry. These areas are frequently challenging for developers and are often critical to process performance.

EB Other analytes, including growth factors, antifoam agents, and other product quality attributes, are also important. These areas require further innovation from suppliers before plug-and-play strategies can be fully implemented and commercialized.

Q What do glucose and lactate measurements indicate in the context of CAR-T cell production?

NR Glucose and lactate are monitored because they directly influence the health and proliferation of CAR-T cells. Additionally, literature has shown that glucose consumption, lactate production, and the overall metabolism of CAR-T cells can impact their efficacy after patient administration. Some studies [5] reported that when low-level glucose control was applied to limit CAR-T cell metabolism, it resulted in higher efficacy upon injection into patients.

EB Glucose serves as the primary carbon and fuel source for cell proliferation, making it critical during manufacturing. Lactate, while sometimes consumed, can also act as an inhibitory component. Therefore, monitoring both analytes in tandem is essential to ensure healthy production.

Q What is the application of PAT tools in a GMP environment? What challenges do they face during GMP implementation?

EB In a GMP environment, there is a strong emphasis on reducing complexity and risk to ensure drugs are produced consistently and safely. However, PAT technologies are sometimes perceived as adding complexity. This perception is not entirely accurate, as plug-and-play PAT solutions can enhance process control, reduce deviations, and consequently lower overall complexity and risk. Shifting the GMP mindset regarding PAT adoption remains an ongoing challenge.

NR While PAT tools are highly valuable in process development for optimization, the goal is to implement these processes and controls within

GMP environments. Typically, manufacturers validate performance during process development for particular modalities and then transition to pilot-scale GMP operations to establish confidence. In the case of MAVERICK, experience has shown that this process is somewhat easier than with traditional Raman spectroscopy, as MAVERICK does not rely on a 'black box' model, which can be challenging to validate and often requires repeated testing. MAVERICK is a commercial off-the-shelf system and is therefore treated like any other analytical technology, such as a pH probe or offline measurement system.

Q What is the impact of PAT tools with intensified bioprocessing?

EB As bioprocessing becomes increasingly dynamic through intensified processing, a wider operational range of cell culture attributes is observed. Greater glucose consumption, increased protein production, higher flow rates, and elevated viable cell densities are examples of these wider operational ranges. This necessitates a more dynamic and robust control strategy, which is precisely what PAT tools, such as MAVERICK, MAVEN, and REBEL, are designed to provide.

Q Where can I get more information about these products?

EB If you would like to request more information about our latest advancements in Process Analytical Technology (PAT) solutions including MAVEN®, MAVERICK® and REBEL®, please request a **Rep Contact from Repligen** or visit **repligen.com/process-analytics**.

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From manual to automated

Building scalable workflows for autologous CAR-T cell manufacturing

Edwin Stone
CEO | Cellular Origins

Mojtaba Parvizi
Global Fast Trak™ Director, Cell Therapy | Cytiva

Matthew Hewitt
Vice President, CTO Manufacturing Division | Charles River Laboratories

Automation and digitalization can enhance scalability, consistency, and efficiency in autologous CAR-T cell therapies, but adoption must be carefully phased and aligned with operational goals. In October 2025, *Cell & Gene Therapy Insights* assembled a panel of leading experts, including Edwin Stone (CEO, Cellular Origins), Mojtaba Parvizi (Global Fast Trak™ Director, Cell Therapy, Cytiva), and Matthew Hewitt (Vice President, CTO Manufacturing Division, Charles River Laboratories), to discuss how automation can overcome challenges in autologous CAR-T cell manufacturing, including patient variability, labor-intensive workflows, supply chain issues, and scalability. This interactive infographic summarizes key insights, including a phased automation roadmap, speaker soundbites, and guidance on balancing timing, cost, and scalability.

Addressing challenges

Click sections to learn more

A decade ago, conversations around autologous CAR-T cell therapies centered on how to reach commercialization. Today, with several therapies now on the market, developers are focused on scaling production to meet growing demand. Manufacturing sufficient doses for patients remains a significant challenge – one that fully automated systems are designed to overcome. By automating both the production process and the scaling of manufacturing, the industry is poised to advance in transformative ways.

> *[Automation is about] revolutionizing this industry by avoiding that manual data entry and PhD-qualified scientists handling manual tubes*

– Edwin Stone Listen

Phased automation roadmap

1. Process establishment and standardization
2. Introducing automation
3. Digital integration
4. Scaling and harmonization

> *Generally, the discussion is not about whether we need the automation, it is about when to implement the automation.*

- Matthew Hewitt

> *Phase automation is not just about the technology; it is also about building the scalability without really sacrificing the flexibility of your process, and also making the compliance and the regulators happy with your documentations and the data.*

- Mojtaba Parvizi

Balancing timing, cost, and scalability

Early clinical phases

Late clinical phases

Late clinical phases

At later stages, such as the commercial scale, the focus shifts to selecting end-to-end automated platforms with global harmonization. This enables training and technology transfers between different sites and helps reduce variability. A key challenge, however, is the diversity of equipment – each device differs subtly, whether in tubing sets, interfaces, or other components. Currently, no standard exists, though establishing one is essential.

In essence, the key principle is to introduce automation at the right stage, select technologies that align with phase-specific goals, and establish a digital backbone that supports both scalability and regulatory compliance.

Aligning automation with regulatory strategy

Oftentimes, the regulatory framework is seen as simply a bar that developers must jump over. However, particularly in the context of automation, regulation and automation should be seen not as opposing forces, but as elements that can strengthen each other.

Operational concerns

Discussions with regulatory bodies about automation often focus on therapy costs, manufacturing expenses, and patient access. While automation typically involves significant upfront investment, a thorough evaluation that accounts for reduced manufacturing failure rates and potential additional revenue can reveal an attractive return on investment.

Long-term value

- Consistency
- Return on investment
- Robust operations
- Reliability

Essentially, regulators should be viewed as stakeholders in this journey, and it is important to understand what drives their priorities.

Exploring the future of autologous CAR-T cell manufacturing

The future of CAR-T cell manufacturing lies in smarter, more connected, and scalable systems that combine physical automation, digital integration, and data-driven intelligence. Scaling up manufacturing will require both technological innovation and a shift toward decentralized, modular manufacturing models. Implementing robotics, automated systems, and advanced analytics can reduce variability and produce more consistent products, ultimately increasing both the quality and accessibility of cell therapies.

Lastly, scaling CAR-T cell manufacturing cannot be done by one party alone – collaboration between cell therapy developers, CDMOs, technology providers, and regulatory bodies is crucial in order to bring these life-saving therapies to patients.

> *I think the future is bright. The clinical data is there, and we have to innovate our way towards the scaling. We have a good or a bad habit, depending how you look at it, of usually innovating our way out of these problems.*

- Matthew Hewitt

Summary

In order to address challenges in autologous CAR-T cell therapy manufacturing, such as variability between patients, labor-intensive processes, and supply chain complexities, automation can be employed to standardize workflows, reduce manual touchpoints, and improve batch consistency, while also supporting scalability and cost efficiency. A phased automation approach ensures that automation aligns with the clinical stage and regulatory requirements. Furthermore, automation and regulatory compliance should be seen as complementary, with regulators engaged as stakeholders to demonstrate benefits such as enhanced product consistency, patient safety, and operational reliability. Overall, to improve both the accessibility and quality of autologous CAR-T cell therapies, it is essential to consider implementing automation, robotics, and data-driven systems, while fostering collaboration among therapy developers, technology providers, CDMOs, and regulatory bodies.

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Closed-system process optimization for CGT manufacturing and storage

Erik Woods, Jon Pileggi, and Sean Werner

The manufacture of cell and gene therapies (CGTs) faces persistent cost, quality, and contamination challenges from open, manual processing and uncontrolled storage. Transitioning to closed-system manufacturing and cryostorage has been shown to reduce batch failures threefold and manufacturing costs by up to 45%, while tripling throughput and maintaining cell viability. This article summarizes comparative data and case studies demonstrating how process closure supports scalable, contamination-resistant, and economically viable CGT production.

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EVOLVING NEEDS IN CGT MANUFACTURING

Cell and gene therapies have transformed oncology and regenerative medicine but remain limited by manufacturing complexity and cost. Typical batch costs, ranging from approximately US $36,000 to over $100,000 per patient, reflect the manual manipulations required under high-grade cleanroom conditions and the infrastructure needed to maintain asepsis at small scale. These operational constraints drive high cost of goods sold (COGS) and limit scalability, highlighting the need for automated, closed systems that deliver reproducible quality at lower cost.

As the field matures, industrial scale initiatives have demonstrated that standardized donor recovery networks and bone marrow–derived manufacturing platforms can enable reproducible sourcing and large-scale processing of cellular materials. The next step is to extend this level of process control and contamination resistance across manufacturing and storage.

TRANSITION FROM OPEN TO CLOSED PROCESSING: OPERATIONAL & ECONOMIC OUTCOMES

In traditional open processing, manual operations performed under Grade A unidirectional airflow within a Grade B background expose products to contamination risk and limit throughput. Closed systems maintain sealed fluid paths using sterile welds and aseptic connectors, enabling production in Grade C or D environments while achieving equivalent sterility assurance.

Closed processing reduces labor intensity and cleanroom requirements, supporting automated, reproducible operations

with minimal operator exposure. When modeled for identical 1,000 m² facilities, closed systems reduce total facility costs by ~50% and expand throughput capacity up to 15-fold. Downgrading from Grade B to C environments alone yields savings of roughly US $45,000 per suite, while lower HVAC and filtration demand further decrease capital expenditure.

Labor, which represents about 50% of total COGS in open systems, falls to 18–26% in automated closed configurations. Fixed labor distributed across higher batch volumes produces an overall 45% reduction in COGS, with batch-failure rates dropping from ~10% to ~3%. Collectively, these changes deliver simultaneous gains in cost efficiency, throughput, and reproducibility while reducing contamination-related deviation or batch loss (Figure 1).

CASE STUDY: VALIDATION OF A FULLY ENCLOSED CAR-T MANUFACTURING PROCESS

A proof-of-concept study conducted at CTMC in collaboration with BioLife Solutions evaluated a fully closed CAR-T process using CellSeal® Connect vials for starting material containment and CellSeal CryoCases for final product storage. The workflow was benchmarked against a conventional biosafety cabinet process using cryovials under identical expansion and transduction conditions.

Across three donors, T-cell growth kinetics, transduction efficiency, and post-thaw viability were comparable between open and closed configurations. Viable cell counts expanded 33–40× from seed to day 7, maintaining >90% viability (Figure 2A). Transduction efficiencies ranged from 43 to 59% CAR⁺ (GFP⁺) viable cells, with total CAR⁺ cell yield equivalent between container types (Figure 2B). Post-thaw recovery exceeded 94% for all donors (Figure 2C).

The weldable PVC tubing of the CellSeal Connect vials remained intact under cryogenic conditions, although long-term liquid nitrogen stability requires further verification. Eliminating biosafety cabinet transfers simplified facility layout, reduced operator exposure, and maintained reproducibility across donors and runs.

These findings confirm that a fully enclosed manufacturing and storage workflow can maintain product comparability while significantly reducing manual handling and contamination risk.

CONTAMINATION PREVENTION IN CRYOGENIC STORAGE

While open manufacturing steps often attract the most attention for contamination control, cryogenic storage presents an under-recognized risk. A documented hepatitis B virus transmission event demonstrated that liquid nitrogen can act as a contamination vector, transferring microbial and viral material between stored vials. Six patients were infected, and subsequent investigations identified microbial growth

FIGURE 1

Comparative impact of open versus closed manufacturing on cost of goods, labor contribution, and batch-failure rate, based on identical facility and production assumptions.

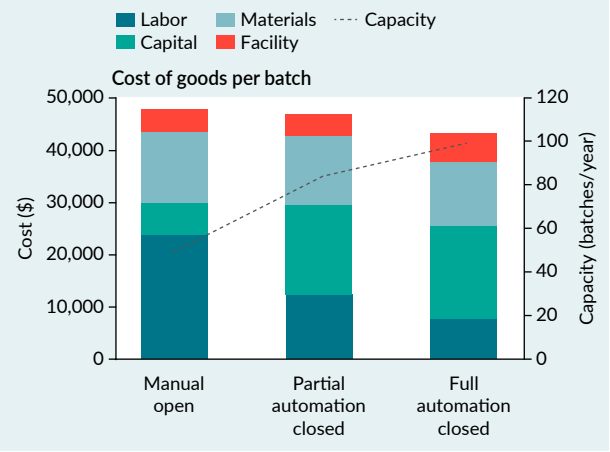
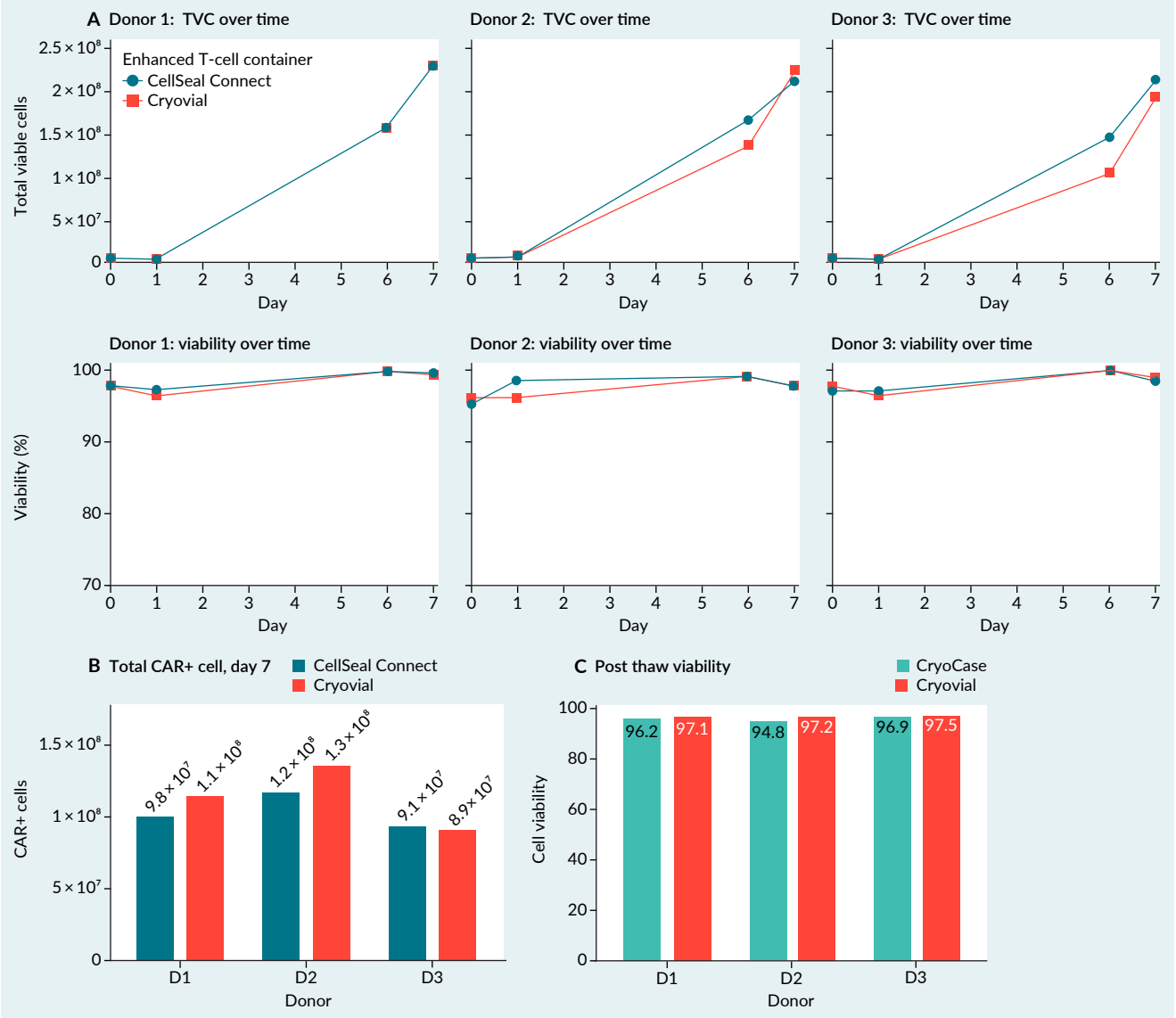


Figure from [1].

FIGURE 2

Comparative outcomes of open and closed CAR-T manufacturing configurations.



(A) T-cell expansion and viability over 7 days for three donors using CellSeal Connect vials versus cryovials. **(B)** Total CAR⁺ cell yield at day 7 showing equivalent outcomes across container types. **(C)** Post-thaw viability of final drug product cells stored in CellSeal CryoCases compared with cryovials.

and human or viral DNA in multiple nitrogen tanks.

Hermetically sealed containers and vapor-phase storage eliminate direct contact between liquid nitrogen and stored products, reducing cross-contamination risk and protecting upstream manufacturing investments. Effective prevention also requires end-to-end validation of shipment and storage conditions to maintain container integrity and sterility. Closed thawing technologies, such as automated, water-free systems for dry thawing, further minimize operator exposure and post-thaw variability.

CLOSED-SYSTEM SINGLE USE TECHNOLOGIES

Single use closed systems have become foundational to compliant CGT

manufacturing, providing validated sterile barriers that minimize environmental control requirements and contamination risk. In upstream workflows, closed bioreactors and bag systems maintain sterility through aseptic welds and verified container integrity. Downstream, disposable chromatography and filtration modules with sterile tube sets support closed transfer during formulation and fill operations.

These configurations sustain sterility without reliance on classified cleanroom environments and align with regulatory frameworks from the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) for Advanced Therapy Medicinal Product (ATMP) manufacturing, as well as inspection standards under the Pharmaceutical Inspection Co-operation Scheme and International Organization for Standardization. Collectively, these standards define expectations for sterility assurance, material qualification, and system validation within CGT manufacturing.

SUMMARY

According to the case studies presented, closed-system integration delivered measurable operational and economic advantages. When applied to equivalent facility footprints, overall manufacturing costs decreased by up to 45%, labor requirements fell from roughly half of total COGS to ~20%, and batch-failure rates declined threefold. Throughput increased up to fifteenfold, reflecting improved utilization and automation.

Empirical data from the closed CAR-T workflow further demonstrates that process closure maintains cell quality and viability while enabling scalable, contamination-resistant manufacturing. Incorporating closed cryostorage completes an end-to-end containment strategy that reduces product loss and safeguards manufacturing investments. Together, these results define a practical, data-driven framework for compliant, scalable, and contamination-resistant CGT production.

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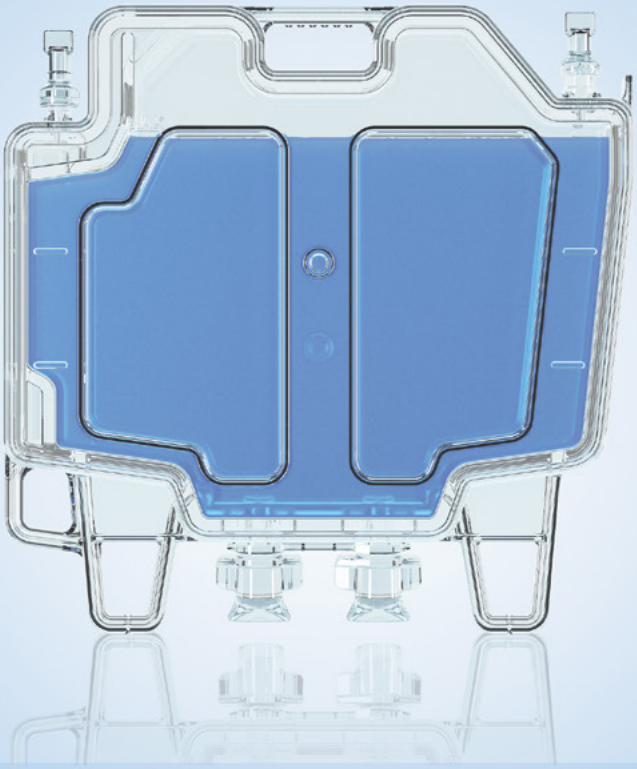

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