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Open or Closed?

How to find the right balance in cell therapy manufacturing

By Krishnendu Khan, Senior Scientist, R&D, at West Pharmaceutical Services

As the demand for cell-based therapies continues to grow, the industry must explore current and future fill-finish packaging strategies. Understanding the advantages and challenges associated with different modes for cell therapy packaging will allow drug manufacturers to choose the most suitable system.

Chimeric antigen receptor (CAR) therapies, as a treatment avenue for various cancers, are gaining in momentum. Current approved therapies are mostly autologous in nature, which ensures no immune rejection of the drug product. However, as demand for CAR-based cell therapies increases, we'll see the current manufacturing process become untenable due to its small production scale, high costs, and the time required for each "batch."

These, and other challenges, are pushing scientists to develop a new generation of cell-based therapies that are allogeneic in nature with "off-the-shelf" options. To make such cell therapies accessible, a complete overhaul of manufacturing is needed as current processes are not equipped for large batches.

Autologous CAR therapies are produced through "closed" processing where the drug substance (cells extracted from patients) is isolated in a manufacturing unit that provides a controlled and sterile environment throughout production, formulation, packaging, and storage, as well as transportation. This approach has several advantages, including minimizing the risk of contamination and protecting the drug product from external pathogens.

"Although closed fill-finish is the way the cell therapy industry currently operates, we need to identify other solutions that allow for better scale up of the manufacturing process."

But cost is an issue; such closed systems use containment technology that requires specialized equipment and infrastructure, often leading to higher capital and operational costs. Two approaches are currently followed: i) the use of modular equipment, where each piece of equipment is used for a single unit operation, such as cell isolation following apheresis, genetic manipulation, expansion followed by harvest, and final drug product formulation, or ii) all-in-one, end-to-end equipment that encompasses the entire process and uses single-use consumables.

Both approaches have their own advantages and pitfalls. Irrespective, as manufacturing needs increase (as will be the case with allogeneic therapies), the use of any equipment must be optimized. Moreover, monitoring critical parameters, such as cell viability or cell count, may require additional sampling or sampling ports that can introduce risks of contamination, essentially, "opening" the process.

Adaptability is another issue associated with closed fill-finish. Current CAR cell therapy manufacturing is designed around T-cells – the first (and relatively unchanged) cell type to be used. But the fixed design and infrastructure of closed fill-finish systems limits their compatibility with the evolving cell therapy landscape that requires the use of different cell types, including NK cells and macrophages. Modification or upgrades to the closed system may require additional validation and regulatory approval, leading to delays and increased costs. Moreover, the scalability of closed systems is limited because of constraints in equipment size or manufacturing capacity, and may require significant investments in additional closed systems or facility modifications as demand increases.

Although closed fill-finish is the way the cell therapy industry currently operates, we need to identify other solutions that allow for better scale up of the manufacturing process. To that end, we could consider an open fill-finish process, such as what we see with monoclonal antibodies. A primary advantage of this approach would be its flexibility in terms of scalability for allogeneic therapies. However, open fill-finish comes with inherent risks, such as increased likelihood of contamination, as well as the requirement for strict aseptic techniques, environmental controls, and highly trained personnel.

When discussing fill-finish, we also need to consider the final packaging container – usually a cryo-bag for cell therapy. These are adopted because of their proven use as containers for blood-based infusion products and also their compatibility with closed fill-finish equipment. But there are various challenges associated with cryo-bags, including bag-breakage at ultra-cold temperatures and the problem of dead-volume, which can lead to dosing errors. Moreover, the requirement for additional packaging material, like over-wrap bags and aluminium cassettes, along with racking systems for storage and transportation, increases the overall price and complexity.

One alternative to cryo-bags is rigid vials, which offer many advantages and are compatible with open aseptic fill-finish processes. Rigid vials provide excellent protection and stability for cell therapy products, with the hermetic sealing of vials providing an effective barrier against microbial contamination while helping to maintain sterility of the product throughout its shelf life. Rigid vials are also more suited for freezing, and can withstand long-term storage in the ultra-low temperatures required for cell therapies without affecting vital container closure integrity. Moreover, rigid vials have been used for a long time for other temperature sensitive therapeutics, such as monoclonals, so their use in cell therapy aligns with established industry practices and regulatory requirements facilitating the approval process. The compatibility, real time monitoring capabilities, sterility assurance, process development benefits and precedence of regulatory acceptance makes rigid vials well-suited for open fill-finish of cell therapy drug products.

The decision isn't simply open or closed; it may also be possible to adopt a hybrid approach, where earlier steps of manufacturing are kept closed whereas the final fill-finish steps are done aseptically to gain the best of both worlds.

The choice between closed and open fill-finish for cell therapy drug products ultimately involves a careful balance between safety, accessibility, efficacy, and the type of cell type used. Closed manufacturing and fill-finish offers robust protection against contamination and environmental factors ensuring integrity of therapeutic cells; open aseptic fill-finish on the other hand provides greater flexibility and a route to scale up, which will be critical in the future as demand for therapies grows. By leveraging the advantages of both approaches, researchers and clinicians can optimize the safety, accessibility, and efficacy of cell therapies.

DIGITAL TRANSFORMATION

Advanced Therapies, Archaic Hardware: the Perils of Paper

Here's how paper is weighing down the future of advanced therapies

By Matt Todd, Head of Digital and Data at Ori Biotech

The phrase "death by a thousand paper cuts" can be aptly applied to advanced therapeutics manufacturing.

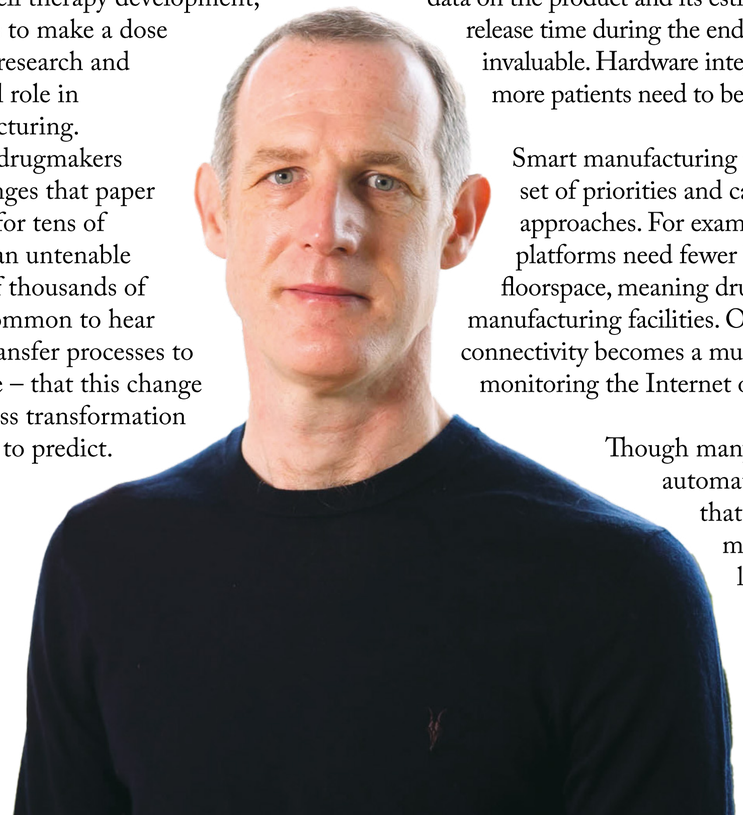
Makers of the first wave of cell therapies leveraged existing processes to reach patients as quickly as possible. And that meant following a path based on manufacturing approaches that are efficient for scaling up large batches of small molecules or monoclonal antibodies, rather than applying bespoke processes to individual patient-derived cells for each therapeutic dose.

Multiple autologous cell therapies are now on the market, but there remain many opportunities in other therapeutic areas. But there are obstacles to reaching patients in need; namely, the high costs, long timelines, and large manufacturing facilities needed to make cell therapies. One lesser discussed limitation is the use of paper-based records. A 1,000-page batch record isn't so daunting when it represents millions of therapeutic mAb doses, but it's a different story when the same type of paper record is required for each dose of an autologous cell-based therapy. After completing a commercial clinical dose, the batch record must be stored in a secure, fireproof cabinet until a document management company collects and stores it for years.

Paper batch records present significant obstacles to obtaining critical data insights, which are essential for accelerating process development and enhancing quality assurance in manufacturing. The inherent inefficiencies and lack of real-time data access delay decision-making and hinder the ability to quickly identify and rectify process deviations. Moreover, the manual nature of paper records increases the risk of errors and complicates the task of ensuring compliance and traceability across multiple production cycles and facilities.

A combination of digitization and integrated hardware is key to cutting the paper out of cell therapy manufacturing. Digitized data can easily be aggregated and used to refine processes. It can be integrated across different steps of the process, accessed across geographically disparate sites, and – crucially – shared with partners. Collaboration remains a critical part of cell therapy development, particularly for reducing the time it takes to make a dose and get it back to patients. Cloud-based research and development platforms will play a critical role in industrializing advanced therapy manufacturing. During the early stages of development, drugmakers often don't recognize the scope of challenges that paper represents for scale-up. What may work for tens of patients in an early-stage clinical trial is an untenable obstacle for a field aiming to treat tens of thousands of patients per year in the near term. It is common to hear early-stage developers say they plan to transfer processes to digital in time, but most realize – too late – that this change is not a minor consideration. It is a process transformation – and most of the challenges are difficult to predict.

Attempting to squeeze digitization into more mature workflows tends to add rather than remove complexity; building it in from the beginning is crucial to ensuring a smooth, sustainable scalability.



Moving away from paper also means automating and integrating connectivity into manufacturing technologies. In many fields, the Industry 4.0 trend of smarter machines improves efficiency and productivity in several ways; for example, making it clear when preventative maintenance is required. When a batch takes weeks to produce and where a single failure can mean life or death for the patient, equipment uptime is critical.

Especially for autologous cell therapies, complex supply chains are required to ship patient biological material from hospitals to manufacturing sites and back. In a paper-based system, manufacturing can be a black box, meaning doctors do not have the necessary information to make key decisions on patient care in the moment. Given that clinicians are managing patients in critical care, having access to data on the product and its estimated time of arrival, quality, and release time during the end-to-end manufacturing process can be invaluable. Hardware integration will be even more important as more patients need to be served at more distributed sites.

Smart manufacturing requires early investment in a different set of priorities and capabilities than today's common approaches. For example, new closed and automated platforms need fewer human operators and less cleanroom floorspace, meaning drugmakers might not need large manufacturing facilities. On the other hand, robust internet connectivity becomes a much higher priority for maintaining and monitoring the Internet of Things-enabled device fleet.

Though many drugmakers wait to think about automation and smart manufacturing, those that adopt and initiate them early will see more significant impact. Early adoption lays the groundwork for resilient manufacturing and logistics models, robust and streamlined scale-up, and the flexibility to constantly learn and improve.

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INTERVIEW

A Guiding Light for CRISPR

The story of how Caribou Biosciences developed a hybrid RNA-DNA guide to increase Cas9 specificity – and what the name of the technology has got to do with the Burgundy wine region in France…

CRISPR has captured imaginations and investor interest, with a growing number of companies now developing therapies based on genome editing. We've also seen the world's first approvals for a CRISPR/Cas9 edited medicine (Casgevy; approved by the UK's MHRA in November and by the US FDA in December 2023). Caribou Biosciences has been working in the area for over a decade and has attracted considerable attention because of its CRISPR chRDNA technology, which can improve the precision of genome edits and reduce off-target events. The company was founded by Rachel Haurwitz (CEO of Caribou) and Jennifer Doudna (joint winner of the 2020 Nobel Prize in Chemistry for her work in gene editing with Emmanuelle Charpentier).

Here, one of Caribou's earliest hires, Paul Donohoue (now Director of Platform Discovery), gives us insight into the early days of the company and how the technology was developed.

How did your experience at the University of California Davis influence the early part of your career?

I grew up in Davis. When I was in high school, one of my science teachers had contacts with a lot of labs at the university and had convinced them to take on undergrad interns. Through this program, I ended up at UC Davis in the lab of Dave Wilson, who was a structural biologist. Dave paired me up with a postdoc student, Eric di Luccio. Eric taught me the fundamentals of science, from how

"A lot of our initial designs had comparable activity to the normal all-RNA guide. It was a eureka moment. We called these hybrid molecules CRISPR hybrid RNA-DNA – chRDNA for short – pronounced "Chardonnay" (remember I had previously worked in the wine industry!)."

to pipette to molecular biology, cloning, protein expression, protein purification, and some early nuggets around X-ray crystallography and structural biology.

It was really challenging but I was really into the work. It was satisfying to work on super hard problems, such as trying to get E. coli to express a human protein and then purify it to the level that it could be used in solving the protein structure. From these challenges, I learned to appreciate simple things, such as seeing a single clean protein band on an SDS-PAGE gel at the end of a purification process.

I applied to attend college at Davis, and Dave also offered me part-time paid work in the lab. It was basic stuff, such as washing dishes, preparing media, and buffers, but I was also able to continue with protein research with Dave. I ended up working in the lab for the next two years of my undergraduate degree, learning more about protein biochemistry and structural biology. As that wrapped up, another principal investigator, Irwin Segel, who had heard about me from Dave, offered me work in his lab.

Irwin was another formative mentor for me. He had been involved in science for decades and had written one of the earliest books describing enzyme kinetics back when it was a nascent discipline. He was not one to suffer people who weren't driven or scientifically curious. He really imparted a lot of those values on to me, and he also imparted to me an understanding and appreciation of enzyme kinetics, which complemented the structural biology insight and protein chemistry I had learned from Dave's lab.

I was very fortunate to have these opportunities and valuable mentors who were invested in me so early on.

Did you join the pharma industry straight out of university?

No – I went into the wine industry! I was really interested in the applied side of science – and at the age of 21 I was developing a burgeoning interest in wine. I ended up in a science job for the Kendall-Jackson Winery. It was a really informative experience, but it wasn't the type of scientific environment that inspired me. Having that exposure redirected me back to an early research focus and I then went into biofuels, which was fascinating – until the company shut down.

While job hunting, a recruiter reached out about a biotech position researching CRISPR in relation to an opening at Caribou Biosciences. I started reading CRISPR papers from Caribou's CEO, Rachel Haurwitz, who had worked in the lab of Jennifer Doudna – Nobel Prize winner in Chemistry in 2020 for her work on CRISPR. Many of Rachel's papers were structural biology based; she was solving the protein structures of Cas proteins, and then coupling this knowledge with fundamental enzyme kinetic characterization of the Cas protein. It reminded me of my work with Dave and Irwin, and I decided this was the environment I wanted to be in.

At the time, Caribou was in an incubator space with just three employees; Rachel, Andy May as the Chief Scientific Officer, and an undergrad intern, who was a computational biologist. Andy was also a structural biologist and, during the job interview, we spent a lot of time geeking out about x-ray crystallography and protein chemistry, and how CRISPR systems worked and could theoretically be applied. There was a lot of energy and excitement – and I was hired for the role. This was back in 2013 and I'm still with the company today.

What is the story behind chRDNA?

In the early days of Caribou, we focused on understanding the basic functional properties of CRISPR-Cas systems and how we could better control their gene editing function. We were particularly interested in understanding the interaction between the Cas9 protein and its guide RNA. Guide RNAs are really interesting molecules because they have lots of secondary structures. We wanted to figure out what elements of the secondary structure were important for driving Cas9- targeting of DNA.

We performed a lot of structural mutations in the guide RNA, including truncating the secondary structures, making them bigger, making sequence changes, and even outright deleting some of the secondary structural elements to see how it impacted the Cas9's ability to target DNA. In time, we understood what parts of the guide were the most important in allowing the Cas9-guide RNA complex to carry out its function.

Something that was interesting to me as I looked at the way the Cas9 protein interacted with the guide RNA was that there wasn't much

direct readout of the 2' hydroxyl group on the sugar backbone of the guide RNA backbone by the Cas9 protein side chains. So I started to wonder whether the guide RNA had to be all RNA? Could we go in and replace some of these RNA bases with DNA?

Andy and I decided to try it. We put together some initial designs of hybrid guides that had DNA and RNA, and we ran biochemical cleavage assays against target DNA. I ran the first one with a large collection of these hybrid guides. When I got the data back, I sent a cheeky email and a summary to Andy for him to review – because I thought the results looked great! That night, Andy responded with excited expletives. Suffice to say, he thought it looked great too!

A lot of our initial designs had comparable activity to the normal all-RNA guide. It was a eureka moment. We called these hybrid molecules CRISPR hybrid RNA-DNA – chRDNA for short – pronounced "Chardonnay" (remember I had previously worked in the wine industry!).

As we worked more with these hybrid guides, we also stumbled across some unique properties they had over the all-RNA system. With CRISPR systems, you program the guide RNA to direct Cas9 to a DNA target sequence, but there are some liabilities. The Cas9 protein can bind to and cleave at target DNA sequences that look similar to the intended target site – in other words, off-target sites. Because of this, using CRISPR systems to edit a human cell can pose a risk. You don't always know what off-target sites might be hit and how this will impact cellular function.

To maximize CRISPR genome editing impact, and ensure its safe use, we wanted to find ways to mitigate off-target effects – and this is where chRDNAs began to truly shine. Through a combination of both DNA and RNA bases, chRDNAs have a very discriminant activity against off-targets. Depending on where we put the DNA bases within chRDNA, we could tune the specificity of the system. In other words, we could build bespoke chRDNAs for each target site.

How is Caribou using the chRDNA technology now?

The complexity of the projects has changed over time, from research of CRISPR tools to development of allogeneic CAR-based cell therapies. For our first clinical program, CB-010, for treatment of B cell non-Hodgkin lymphoma, there are three edits (two gene knockouts and one gene knock-in). In our second program, CB-011, for treatment of multiple myeloma, we make four edits. Our newest program, CB-012, for treatment of acute myeloid leukaemia (AML), involves five edits. As we make more edits, we need a system to help accomplish that with maximum efficiency and safety, such as the chRDNAs.

One of the first patients in our non-Hodgkin lymphoma trial had eight prior lines of treatment before being put on our clinical trial. With a single dose of CB-010, our off-the-shelf CAR-T cell therapy, he has been cancer free for two years. It's incredible to see the impact that these therapies can have for patients. Out of 16 patients we treated in the dose escalation portion of our CB-010 ANTLER phase 1 trial, 44 percent are cancer free out to six months and beyond. It's very humbling to see how our science has directly impacted patients. I joined Caribou because I thought CRISPR proteins were interesting and today it has evolved far from what I imagined.

How has your role at Caribou developed over the years?

Today, I'm an associate director and I lead a small team of highly motivated, very bright researchers. My role is about passing the baton on and relying on my team to come up with new ideas that will drive further innovation in CRISPR systems and how we use them.
In addition to learning to be a manager and a leader, I've had to develop a broader understanding of the biotech space and how a biotech company is run. Sometimes I have to get involved with our business development team and speak to potential partners about our technology, and I've also had opportunities to get involved with our clinical team, talking with clinicians or nurse practitioners about cell therapies. I've also had to interact with our legal team about IP and patents. It has all been very interesting.

Running a team now has also made me reflect on my time at Davis in terms of my early mentors and how I can mentor others. It's important to find the best way to motivate people and to maximize their scientific creativity.

What keeps you excited about the future of CRISPR?

It's been really shocking and surprising to see the rapid implementation of CRISPR across all areas of science. Everything has moved very fast; we're now using CRISPR for cell therapies and for genome editing plants and eukaryotes. It's almost ubiquitous and is already having medical impact with the first approval of a CRISPR-based therapy in the UK coming after just over a decade after the seminal work on CRISPR-Cas9 genome editing by Jennifer Doudna and Emmanuelle Charpentier and their colleagues. It's amazing to take a step back and appreciate how much has changed now that we can make genetic manipulations.

I will never forget when we interviewed a scientist out of his postdoc. He gave a presentation about his work where he had been trying to tag a mouse neuronal protein with a fluorescent reporter. He spent almost a year of his postdoc trying different approaches, but he finished his presentation by saying that the work could have probably been done in two months now that CRISPR-Cas9 was a tool at researchers' disposal. It really encapsulates how much CRISPR has changed things for the scientific community.

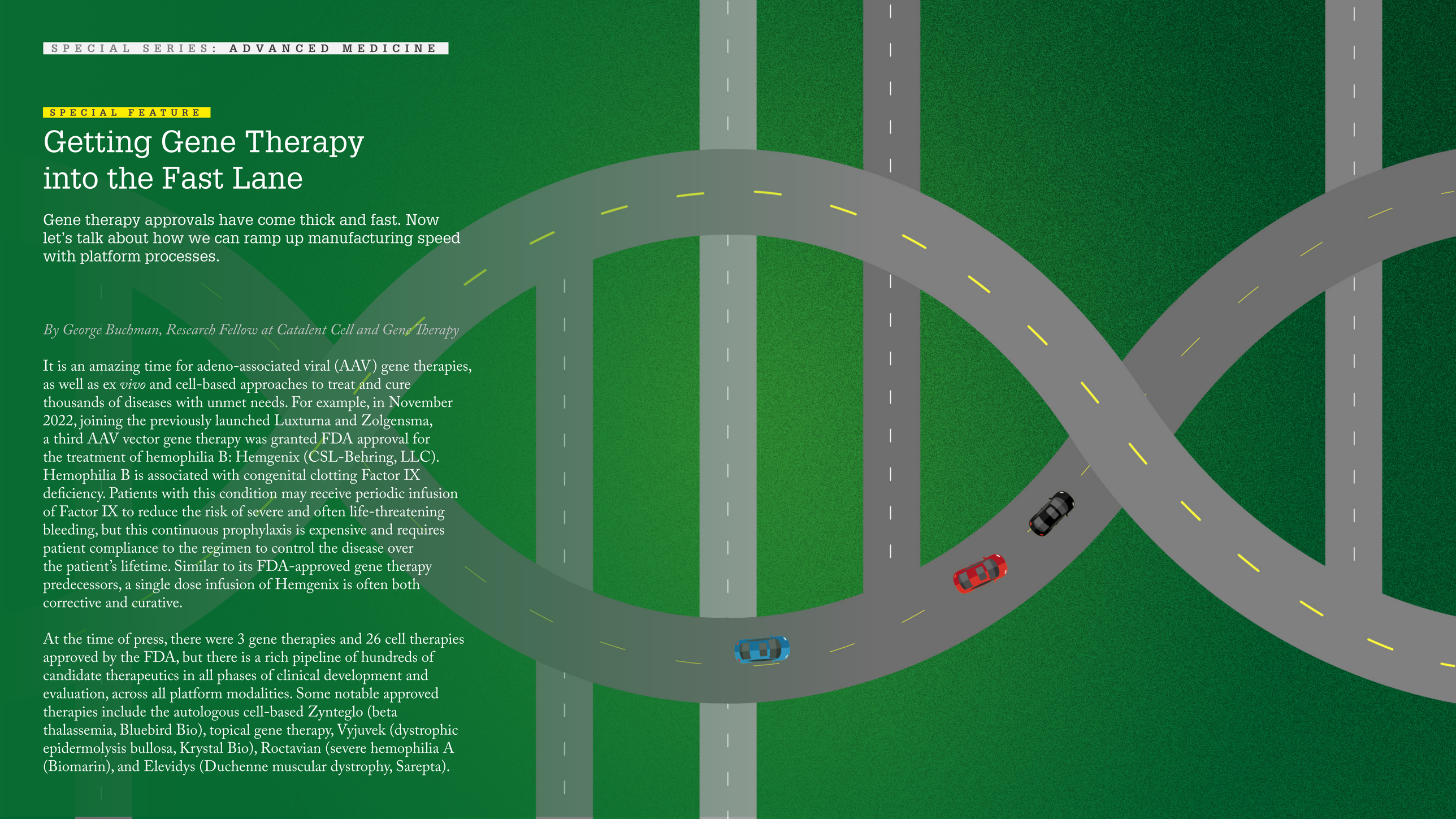
And yet it's still early days. There are many new ways that we can push what we're able to do with CRISPR systems and how our understanding and deployment of them evolves.

FULL ARTICLE AVAILABLE ONLINE

SPECIAL FEATURE

Getting Gene Therapy into the Fast Lane

Gene therapy approvals have come thick and fast. Now let's talk about how we can ramp up manufacturing speed with platform processes.



By George Buchman, Research Fellow at Catalent Cell and Gene Therapy

It is an amazing time for adeno-associated viral (AAV) gene therapies, as well as ex *vivo* and cell-based approaches to treat and cure thousands of diseases with unmet needs. For example, in November 2022, joining the previously launched Luxturna and Zolgensma, a third AAV vector gene therapy was granted FDA approval for the treatment of hemophilia B: Hemgenix (CSL-Behring, LLC). Hemophilia B is associated with congenital clotting Factor IX deficiency. Patients with this condition may receive periodic infusion of Factor IX to reduce the risk of severe and often life-threatening bleeding, but this continuous prophylaxis is expensive and requires patient compliance to the regimen to control the disease over the patient's lifetime. Similar to its FDA-approved gene therapy predecessors, a single dose infusion of Hemgenix is often both corrective and curative.

At the time of press, there were 3 gene therapies and 26 cell therapies approved by the FDA, but there is a rich pipeline of hundreds of candidate therapeutics in all phases of clinical development and evaluation, across all platform modalities. Some notable approved therapies include the autologous cell-based Zynteglo (beta thalassemia, Bluebird Bio), topical gene therapy, Vyjuvek (dystrophic epidermolysis bullosa, Krystal Bio), Roctavian (severe hemophilia A (Biomarin), and Elevidys (Duchenne muscular dystrophy, Sarepta).

"With potentially thousands of genetic diseases treatable by gene therapies, the possibilities are nothing short of staggering and we can expect to see a need for higher manufacturing capacity and volumes in the future."

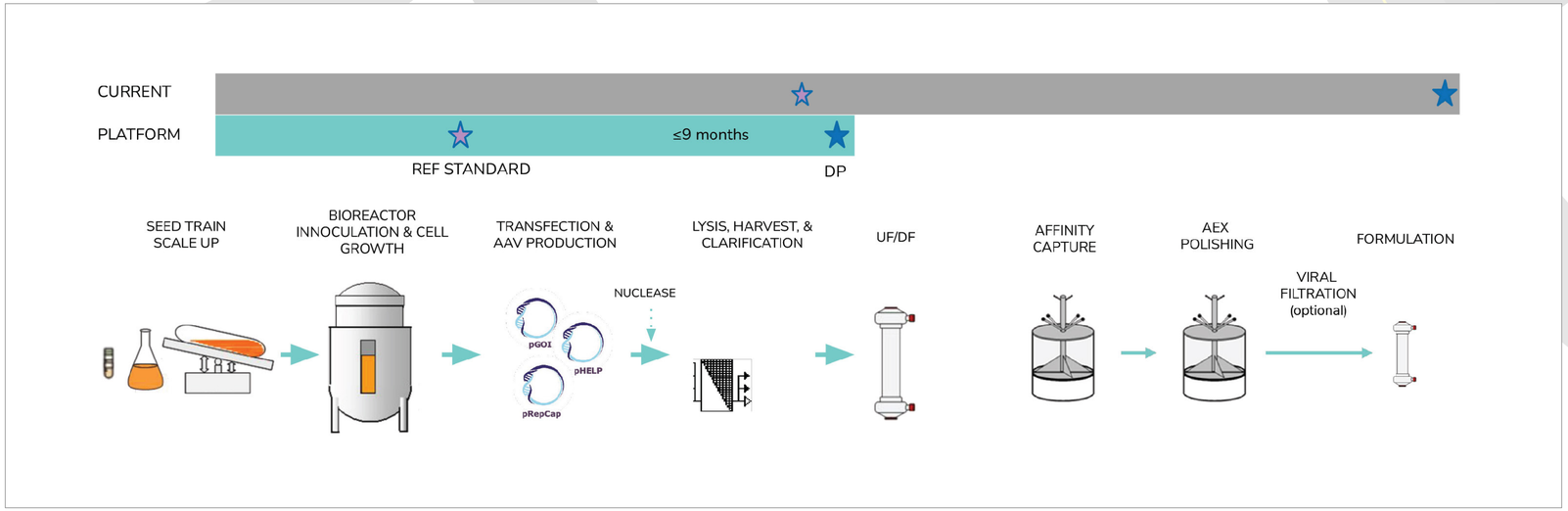


Figure 1.

In 2023, we also saw approval of Casgevy, an editing technology for beta-thalassemia and sickle cell disease (CRISPR Therapeutics). Among the hundreds of early stage clinical candidates, UX701, a gene therapy for Wilson's disease (Ultragenyx) and RPL102 (Rocket Bio), a lenti-based ex vivo gene therapy indicated for Fanconi anemia are among the many candidates vying for late stage clinical and ultimately regulatory approval.

Creating a platform approach

With potentially thousands of genetic diseases treatable by gene therapies, the possibilities are nothing short of staggering and we can expect to see a need for higher manufacturing capacity and volumes in the future. For the present, however, these therapies typically target very niche patient populations and are not front-line treatments. Patients that are eligible for a gene therapy or that take part in a gene therapy clinical study are typically quite ill, face comorbidities, and may be experiencing side effects from standard of care treatments. For these reasons, speed to first-in-human examples for new therapies is crucial; delays in reaching the clinic can cost patient lives.

There is a perception that the development of a gene therapy is a complex process. In many ways this is true, but the development of processes and analytics, as well as the manufacture and release of product, can be simplified and standardized to a large extent by using a platform approach incorporating single use and scalable equipment. In a platform approach, time savings can be realized in all steps of the development and manufacturing processes; from project launch, to rapid and focused development studies, and engaging a templated transfer to the manufacturing and quality control (QC) groups. For example, a standardized process may include the use of consistent consumables, buffers, and media, which helps to simplify the supply chains, and means that materials can be stocked in advance. Developers can also benefit from templated master batch records and standardized test/release methods. Templated manufacturing records and platform release assays, for example, dramatically reduce the time to review and approve methods, as well as simplifying training, and reducing errors in GMP production. In short, the GMP, QC and quality assurance groups know what to expect, and are able to execute efficiently and with minimal error or delay, leading to faster and higher quality delivery of therapies to the patient.

An example of a platform approach is graphically described in Figure 1. The platform approach comprises, in part, early, well-documented production of pre-clinical viral vector lots in the non-GMP development laboratory. These materials are produced from a locked and (preferably) scaled process on equipment comparable to that used in GMP manufacturing. Vector lots for preclinical toxicology or efficacy may be produced in this way, as well as reference material for assay qualification to support GMP vector release for first-in-human studies. Raw materials should ideally be off-the-shelf and may include a well-performing clonal HEK293 production cell line, and off-the-shelf transfection reagent and plasmids. High quality plasmid is critical to a successful transfection, so extensive QC release testing is needed for these materials.

Table 1: Cell and gene therapies: representative release analytics

Test Scope	Gene-Modified Cell Therapy Products	Viral Gene Therapy Products
Safety	- Viable cell number - Quantitation of specific cell population - Total DNA - Total protein	- GOI quantitation - Virus particle number/Infectivity - Transducing units - Total protein
Identity	- Mycoplasma - Sterility/Bioburden - Endotoxin - Adventitious viruses (in vitro) - Residual virus - Replication competent virus	- Mycoplasma - Sterility/Bioburden - Endotoxin - Adventitious viruses (in vitro) - Replication competent virus
Strength	- Viable cells (%) - Transduced cells (%) - Specific cell surface markers (%) - Process contaminants	- Residual HC DNA - Residual RNA - Residual HC protein - Full:Empty particles - Optical density ratios - Process contaminants - Viral protein profile (HPLC; for defective virus)
Potency	- Viable cell number - Bioassay (colony formation, expressed protein function, etc.)	- Bioassay (RNA expression, expressed protein function, etc.)
Quality	- Cell surface markers - Cell species ID - Morphology - Bioassay - Biochemical markers	- Restriction map - PCR - EIA for expressed protein - Sequencing

Essential to the platform approach is the inclusion of program-specific approaches for optimization. In this phase, process developers will need to conduct focused development (such as using small scale experiments in flasks/parallel bioreactors) on the variables most critical to productivity and quality/safety, such as transfection reagent selection and plasmid molar ratio. Here, in-process analytics are critical to verify expected vector titers and quality as early in development as possible; it is much easier to correct problems at the development stage than it is in manufacturing. Key in-process analytics include vector genome titer (ddPCR, dPCR), total capsid (ELISA), encapsidated hcDNA (qPCR), vector protein profile (SDS-PAGE), and fidelity of vector packaging/percentage of full AAV particles (analytical ultracentrifugation).

For a locked platform process, the corresponding process and release analytics should be locked in when manufacturing is reached.

SIDEBAR

Overcoming the Immune Barrier

As well as the development of more platform-based approaches to development and manufacturing, other innovations are also being seen in the gene therapy space. Novel capsids, for example, can improve targeting to the correct tissue, as well as direct delivery (as opposed to systemic) to the target tissue or organ, reducing dosing requirements and potential toxicity.

Some of the greatest barriers to AAV-based theories are pre-existing immunities to the AAV capsid. The vector itself can also invoke both humoral and cellular deleterious responses that may cause inflammation or other toxicity. Further, in the current state, re-dosing with the same AAV vector is not possible due to the immunity generated at the first dose. The study of AAV-induced deleterious immune response and possible remedies is an area of tremendous, ongoing study. The literature is rich with reviews and studies of progress toward reducing or eliminating this immune-based barrier. Reduced dosing by improved targeting is one possible remedy, and this may be achieved by direct delivery of the vector to the correct tissue or targeting via modified capsid design (1). In another approach, scientists describe immune response derived directly from the AAV transcription and translation products. The authors contend that pathogen associated molecular patterns (PAMPS) may be modified to reduce deleterious immune response (2). Addressing immune-induced toxicity and inflammation further opens the door to this exciting area of new and curative medicines.

"Catalent is already seeing growing use of platform approaches and expects these to be further optimized as industry experience with commercial gene therapies increases."

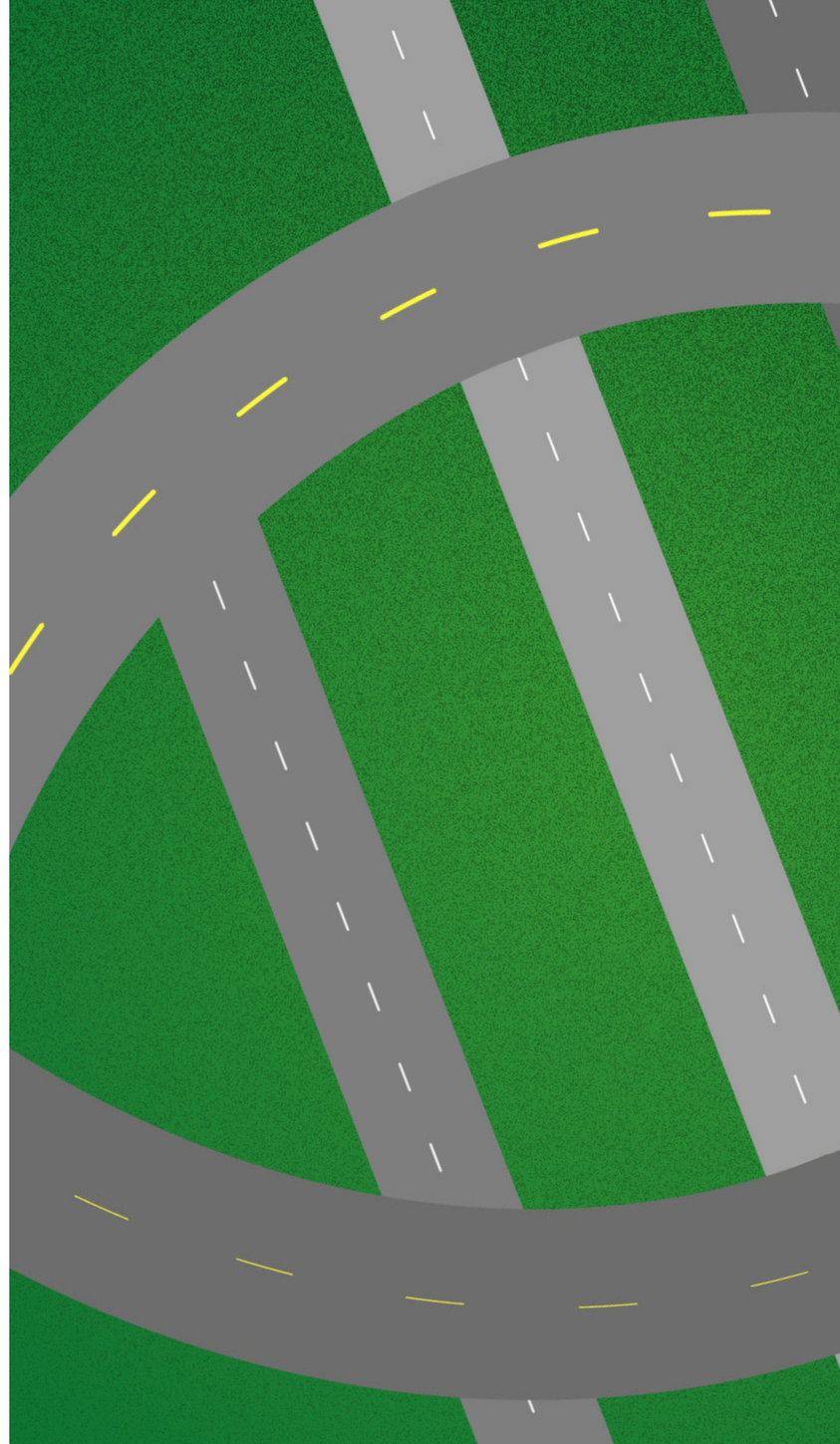
Representative release testing for both cell and gene therapies are summarized in Table 1. As is evident from the table, comprehensive safety and residual testing is needed to measure for vector quality, potency and safety. Encapsulated host cell DNA may present a significant regulatory and safety risk and must be measured and monitored closely. Adventitious viruses may be propagated during cell culture, so in vitro testing for these materials on crude harvest material is used to monitor upstream culture samples directly.

The road to commercialization

During the development of any gene therapy, developers must keep their methodology focused on the ultimate goal of commercialization. During early development and initial clinical evaluation, it may be too early to make substantial investment in commercial activity pending early clinical outcomes. However, the foundation should be set in these early stages to enable seamless transition to late-stage studies and, in due time, regulatory submission. Platform methods would mean that the commercial teams are already informed of the key materials, which they can stock and qualify; batch procedures can be templated and staff trained; and release testing can be validated. AAV viral vector development and manufacturing will still vary between each project (even swapping a transgene in an otherwise-locked process may give differing yields and quality), but overall a platform approach will help the manufacturing and quality teams be more prepared. A robust change control process, and thorough training for operators as needed, can also be put in place in anticipation of minor adjustments to the platform.

Thousands of diseases with a genetic basis may be curable with gene therapy approaches. Current barriers include low vector yields and high cost of goods, but continued innovation will eventually bring viral vector development and manufacturing to the state of biologics: monoclonal antibodies are routinely produced in multi-gram quantities per liter using stable cell lines, perfusion and high-density culture. Catalent is already seeing growing use of platform approaches and expects these to be further optimized as industry experience with commercial gene therapies increases.

REFERENCES AVAILABLE ONLINE



DONOR CELLS

Getting Off to a Good Start

Why finding high-quality donor cells is so important in cell therapy development

By Priya Baraniak, Chief Business Officer of OrganaBio

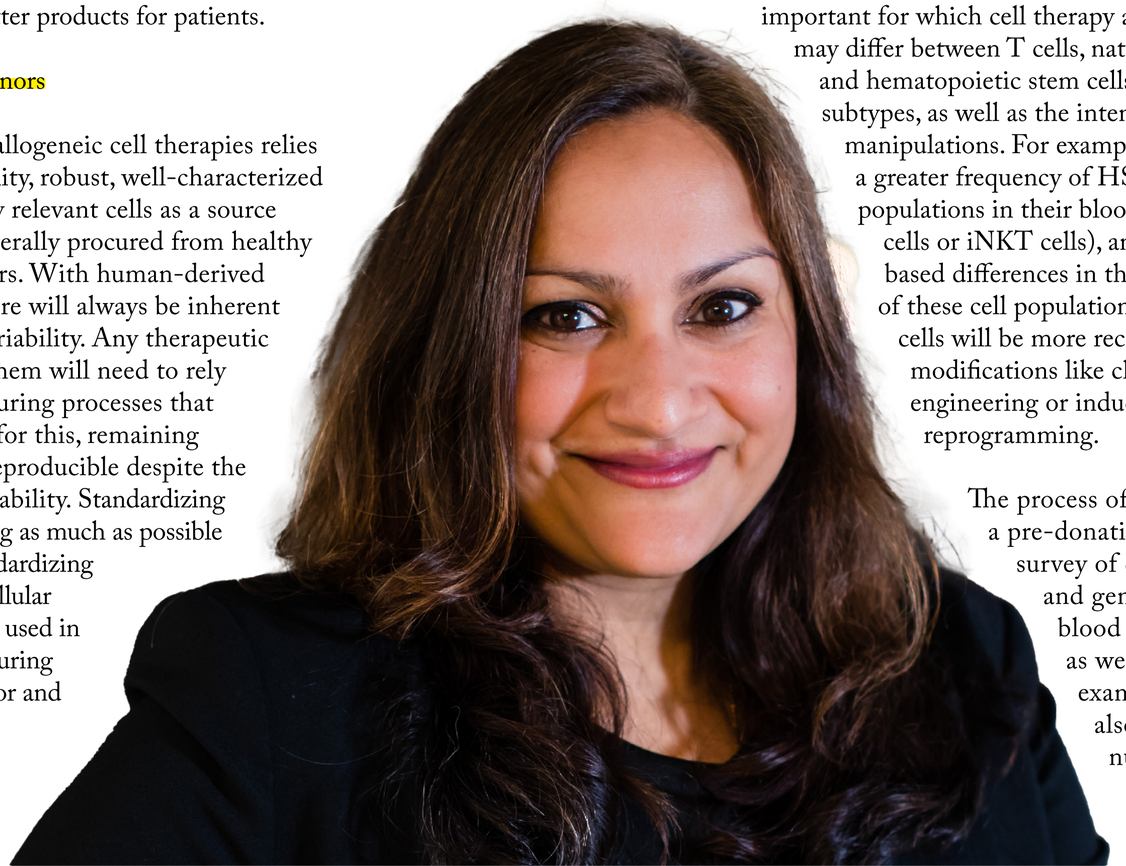
The complexities of making a living medicine from human cells demand that developers of cell therapies consider many variables. And because the industry is still young, much remains to be learned about optimizing processes to ensure the best products – with some standards either lacking or non-existent. The uncertainty results in companies being forced to spend time and resources well outside their core competencies and proprietary technologies, particularly when it comes to starting materials.

From our interactions with developers, we've seen the hope and need to focus on the therapies rather than the starting materials and the intricacies that come with them. The particulars of donor management and tissue sourcing are like an airline pilot learning how flight control systems work. Thus, for far too long, the industry has been complacent about the sourcing and characterization of cellular starting materials, often procrastinating and leaving the issue for later phase development. But something changed. Evolving approaches to interrogating these drug products (and the processes underlying their development) from regulatory agencies – coupled with supply chain disruptions during the pandemic – served as a wake-up call for the future of the industry. We were all reminded that we are still far from sustainability and that strong partnerships across the stakeholder value chain will be critical for success.

Since then, the industry has continued to expand rapidly, with impressive clinical advances by therapeutics developers. With this progress has come a range of enabling tools and technologies. Some, like automated manufacturing platforms and AI, are generating significant attention for their potential to improve scalability, save time, and reduce costs. It is therefore easy to overlook the work needed to secure the supply of critical raw materials, which will remain the backbone of scalable growth upon which the whole field relies. It makes sense that this space needs to mature along with the rest of the cell therapy value chain. By partnering with companies to ensure that the optimal donors and donor-derived materials are procured for their therapies – and educating them in the process, we are seeing better management of the variables inherent to human cell-based therapies. Ultimately, such collaborations will translate to more and better products for patients.

Screening donors

Developing allogeneic cell therapies relies on high-quality, robust, well-characterized and clinically relevant cells as a source material, generally procured from healthy human donors. With human-derived products, there will always be inherent biological variability. Any therapeutic made from them will need to rely on manufacturing processes that can account for this, remaining robust and reproducible despite the inherent variability. Standardizing manufacturing as much as possible includes standardizing the critical cellular raw materials used in the manufacturing process. Donor and tissue screening demands a series of testing, regulatory and ethical requirements, as well as a rising GMP bar to ensure quality and safety as a product moves into clinical trials.



In the context of many cancer immunotherapies, cells are typically isolated from donors' blood via apheresis, where components of interest (white blood cells) are collected and the rest is returned to the donor. Due to physiological differences between humans, the abundance and potency of cells – and their suitability for a particular therapeutic indication – can vary greatly, making some donors better sources than others for cell therapy developers.
More research is needed to understand which variables are most important for which cell therapy approach – and the answer may differ between T cells, natural killer (NK) cells, and hematopoietic stem cells (HSCs), and all of their subtypes, as well as the intended downstream cell manipulations. For example, some donors will have a greater frequency of HSCs or other rare cell populations in their blood (such as gamma delta T cells or iNKT cells), and there will likely be donor-based differences in the ex vivo expansion potential of these cell populations. Similarly, some donors' cells will be more receptive than others to genetic modifications like chimeric antigen receptor engineering or induced pluripotent stem cell reprogramming.

The process of donor selection begins with a pre-donation screening, including a survey of demographic, physiologic, and genetic factors (age, sex, BMI, blood type, and HLA genotype), as well as lifestyle factors (for example, smoking history). It also includes testing for a number of infectious diseases

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as mandated by the FDA and other regulatory agencies. The number of specifications and the requisite testing methodologies can vary depending on the needs of the developer and the relevant regulators. The more specifications and the stricter the regulatory review, the smaller the donor pool. Add to this the typical demographic profile of a donor who donates for money and the pool becomes even smaller.

There are also factors beyond what we know to screen and test for that can influence product quality, meaning that even among donors who meet all specifications, some will donate tissues and cells that will unpredictably be more valuable than others. Changes to donors' health, their lifestyle choices, and something as simple as whether they were exposed to a common cold can all affect their immune cell profile. In addition, because of the highly reactive nature of our immune systems, any immunophenotype analysis is, in reality, just a snapshot in time. Though some donors may be predisposed to a higher abundance of certain cell subpopulations, this is subject to change on any given day, and there's no guarantee that a donor who provided exceptional material at one donation will do so at the next. As such, it is imperative that cell-based therapeutics developers curate a robust donor pool that has enough redundancy to weather changes in donor health, as well as natural attrition on account of donors losing interest in donation, relocating to another area, or any number of other reasons.

Emergent technologies – for example, AI, metabolomics and proteomics screening, and genomic analyses – are paving the way for more effective personalized medicines. These technologies may also lend themselves to predictive means of assessing with better accuracy which donors may be well suited for specific tissue and/or cell donations, increasing the efficiency of donor screening programs and donor pool cultivation. In addition, advances in cell isolation and expansion may also obviate the need for large donor pools, if master cell banking strategies may be applied to cells for which such approaches are not a current possibility.

Planning for sustainability

Finding the right donors is just the first step; as therapies scale into the clinic, we also need to be able to recall donors – a major challenge for the field.

The logistics of recruiting and nurturing a robust donor pool must then include identifying donors who are able and willing to return again and again, as needed, and encouraging them to do so. Apheresis itself takes hours, which can be onerous for some donors. And even for those willing to come back, each person can only donate every so often. According to best practices in the industry, blood collection sites should only apherese donors a minimum of every eight weeks, with donor well-being prioritized and the quality of the collection a close second, since the body needs time to replenish the depleted cell compartments. Thus, determining the number of donors needed for a sustainable cell therapy program must take this cadence into account on top of natural attrition rates.

This complexity can become a problem as a program moves through the phases of clinical trials and into commercialization. Developers often start with one regular donor for process development purposes, but, by human clinical testing, they will often need three to five donors. Beyond absenteeism, donors can have transient issues, such as low iron, fever, infection, or dehydration that prevent them from donating on schedule, requiring a temporary deferral. Thus, even if a company only needs a single leukopak per month, it would require two alternating donors, with suitable substitutes available for unforeseen circumstances. By the time a therapy is commercialized, a stable supply chain might require 100 leukopaks per month, from a pool of 300-500 regular donors to minimize risk of insufficient donors and starting materials for manufacturing campaigns as and when needed.

The specific needs of a developer will guide the donor recruitment campaign itself, which is where strong relationships with collection partners are needed. Collection centers will start from a pool of documented donors (ideally in a detailed database including demographics and genotype information), which will help calculate what percentage of donors are likely to meet the developer's criteria. After accounting for attrition, the collection center will estimate how large the campaign must be and how many known and new donors must be recruited for a single developer's needs. At some point, most developers will need a dedicated donor pool to ensure the sustained success of their program.

In cell therapy, the prevailing wisdom is that "the process is the product," and developers are rightly focused on their novel technologies – optimizing manufacturing processes to make the best therapies they can. Strong partnerships with the providers of their source material can ensure they eliminate as many variables as possible and set the stage for sustainability, smoothing the journey from scale-up to commercialization. And I would argue that establishing such relationships early is critical to long-term success.

SITTING DOWN WITH

Be a Little Different

Sitting Down With… Luigi Naldini, Director, San Raffaele Telethon Institute for Gene Therapy, Milan, Italy

Luigi Naldini has always been fascinated by research. After initially focusing on signal transduction, he became intrigued by the gene therapy field – where the drive to try something new led to the development of lentiviral vectors for use in commercial gene therapy.

How did it feel to receive the Lifetime Achievement Award at Phacilitate 2024?

It was very rewarding – as with any award! Gene therapy has been neglected for so long, but now there is appreciation from all over the scientific industry. Early on, there were very few of us working and believing in what could be done with gene therapy. Now, there is much better recognition. Although an award goes to a single person, that person doesn't deserve all the credit. This award really goes to a whole team of people who have been involved in different stages.

Have you always wanted to be a scientist?

I always loved science, but early on it was more about nature and wildlife. In high school, I became more familiar with the emerging concept of molecular biology. At that time, there was no real understanding of DNA and RNA, so it was like an entirely new world was opening up – I found that very attractive. I ended up going to medical school, which, at the time in Europe, was a common path if you were interested in a research career in the biomedical area. Although I am an MD, I rarely practice or conduct clinical work. I am more interested in basic science and translational research.

How did you get into gene therapy?

After my MD and PhD, I started work on signal transduction. Back then, we were uncovering the basics of growth factor receptor tyrosine kinase, but I wanted to take a new route. I came across a review about the emerging area of gene therapies by Richard Mulligan (Harvard). After the early hype of gene therapies and the lack of results, he explained that we needed to go back to the hard science.

I was attracted by this idea and I wanted to join the field. I went to the US and I applied to Richard Mulligan's lab, but I didn't get the role! Over the years, I became very close to him and he always said, "Too bad you couldn't come to my lab."

And I would reply, "I could have come to your lab, but my application was rejected!" Fortunately, I was also interviewed at the Salk Institute and ended up in the lab of Inder Verma.

Why focus on lentiviral vectors?

At the time, there was discussion around current vectors, such as the gamma retroviral vector, not being very efficient. On the floor above me was the lab of Didier Trono working on HIV. It was early days for HIV and there was a lot of work focused on understanding this deadly retrovirus, which was very efficient at infecting human cells. We thought, why not try creating a vector from HIV? I was interested in starting something from scratch in gene therapy rather than joining something that was already going on, so building a new vector was very appealing. Though we never dreamed it would become so useful!

I worked for two years on this project – and it was very difficult at the beginning, particularly as it was a new area for me. I spent at least a month in the library, browsing literature (which is amazing to think about today, given that you can do that in a matter of days using the internet!).

We tested the lentiviral vector we had developed in the brain of a mouse. Could we prove transfection of a neuron? The "eureka"

moment was when we got that neuron shining with GFP. It was a big accomplishment – and after that I planned to return to Europe. However, a biopharma company was interested in licensing the technology for product development.

I resigned from my position in Europe and began working with the company to develop the vector for clinical trials in humans. However, the whole field came to a halt because there were reports of tumors developing in patients treated with a gamma retrovirus in Europe. Theoretically, we should have anticipated this but there was not high recognition of the risk at that time. Many companies were scared away from gene therapy – including the company I was working with.

At this point, I returned to academia in Italy and I continued to develop the technology on an academic basis – thanks to funding from the Telethon Foundation and other sources. We also collaborated with researchers in France and eventually we took our lentiviral vector into clinical testing and showed that it was safe.

Paradoxically, an HIV-derived vector was safer than the earlier gamma retroviral vectors! We really did work hard to disable the original virus and improve the safety – but the results went beyond expectation. If treated early enough, children can now be cured of very deadly diseases. Our work attracted people back to gene therapy – including big pharma. Together with the Telethon Foundation CEO we spoke with GSK executives and this led to an alliance for the development of hematopoietic stem cell gene therapy. We developed a handful of successful treatments with them, including the first ex vivo stem cell gene therapy approved worldwide.

All of this work took more than two decades.

How did it feel when lentiviral vector therapies made it to market?

Progress doesn't happen in a single moment. Yes, early experiments can have a "eureka" moment but it takes time to bring this to humans. When you see results in patients and the disease doesn't seem to be appearing, you need to wait months before you can be sure of the results. It also then takes time to get to market. But it feels amazing!

The whole experience has been a learning curve for us as well as the industry. I feel very lucky that I've been so closely involved, from the early steps on the bench, to clinical, and then to market. I've also been able to see the challenges from both academic and industry levels.

Where does the industry go from here?

We are finally at the point where gene therapy is an established treatment – but mostly for some rare genetic diseases and some types of blood cancers. There is a big need to streamline the process through industrialization, and to address cost and accessibility.

There is also a lot of hype now around gene editing, which has potentially broader application than a gene addition, but it's early days for understanding its power. Until now, gene editing, to a certain extent, has been leveraging on what had been done with gene addition and addresses the same targets, such as hematopoietic stem cells, T cells, the liver and retina. There is still limited capacity to deal with other targets. Gene editing is great but we still can't target the heart or the brain – because of challenges in delivery, which are just as much of an issue today as they were 30 years ago. There is excitement around nanoparticles and other innovative delivery vehicles, but most of these currently work for the liver. To realize the promise of gene editing, we need to have better targeting in vivo – scientifically, this remains a big challenge.

Going forward, we also need to continue to be careful about the risk–benefit balance for patients. There are now many tools to choose from and patients must be protected from testing innovations that might be moved to the clinic too early. I would like to see new technologies tested in new diseases that have no other options, rather than going for the same indications. Companies prefer the latter option because it is easier for them to see if the new treatment is really better or not. But what if it is not better for the patient? We must be cautious.

Do we need more intense collaboration to move forward?

What we have achieved today in gene therapy is the result of academic research, charity funding (crucial), and involvement of industry – from small biotech to big pharma. While industry was too afraid to commit in the early years, a network of institutions and European grants helped create a community collaborating to the development of cell and gene therapy.

Collaboration must continue – but we also need open transparency. No single treatment or tool is perfect, and there will always be advantages and drawbacks for each of them. If there is a problem with a tool, it is much better to acknowledge that upfront rather than cover it up. There is the risk of the field moving into a more protective, business and venture capital driven model. We have managed to achieve so much today because there has been data sharing and open discussion from the very beginning. Without this, we risk building a culture of suspicion. We must be open about the risks and not oversell the benefits.

What advice do you have for scientists who are just entering gene therapy research?

First of all, I would encourage them to enter this area because it is very exciting. It can also give you a broad translational view and help you understand the rationale behind what we do as scientists. I would also recommend people to spend time in academia because basic science understanding and training is key. Today, most young scientists go to industry because of better salaries, but I believe that the best way of doing this type of science is to maintain a strong focus on the underlying biology and address the real challenges. Spending time doing postdoctoral training in a good academic lab to really understand the key points of the field. From there, you can then look at new strategies and be creative.