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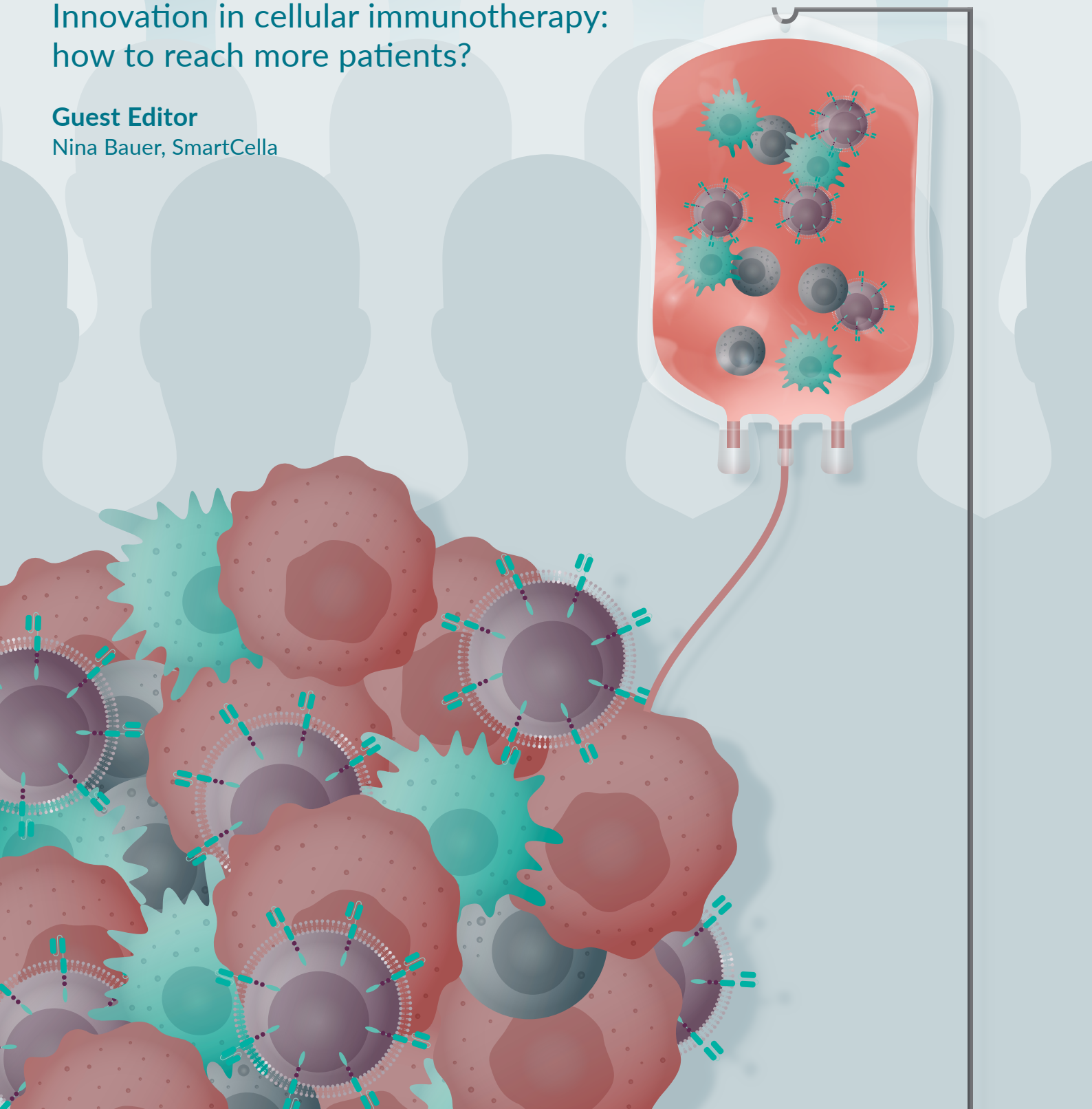
CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON
Innovation in cellular immunotherapy: how to reach more patients?

Guest Editor
Nina Bauer, SmartCella



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Nina Bauer
SmartCella

"...we are approaching an era where our industry will have a solid repertoire of therapeutic modalities...and delivery approaches."

FOREWORD

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It has been a privilege to guest edit this month's *Cell and Gene Therapy Insights* issue 'Innovation in Cellular Immunotherapy: How to Reach More Patients?'. The fact that we can compile this many articles, addressing anything from increasing regulatory guidance to the expanding portfolio of cell types used, indicates that this space is truly maturing, and we are now planning for the long-term.

I'm going to venture a guess: cell-based therapies are here to stay, and more and more patients will benefit from them.

In this issue, our authors touch on expanding into the cancer space beyond CAR-Ts in hematological B-cell malignancies, and the initial successes and ongoing efforts underway to branch out into solid tumors: non-CAR-T cell therapies are approaching the market in the solid tumor arena, including Iovance's AMTAGVI (tumor-infiltrating lymphocytes) and Adaptimmune's afami-cel (engineered T-cell receptor construct), and natural killer cells also remain an area of focus. An interesting ancillary effect of CAR-Ts being approved as second line treatments is that we are gaining a much better understanding of the quality characteristics of healthier cells (from patients that have not undergone series after series of chemotherapy). These insights could be an opportunity to refine pluripotent stem cell-based differentiation protocols with robust quality templates, and thus, potentially, allow us to move to cheaper, scalable, and truly off-the-shelf options. In parallel, numerous efforts are underway to facilitate *in vivo* cellular immunotherapies. If successful, targeted lipid nanoparticles (LNPs) or dialysis-like cell engineering would make treatments much faster and more financially viable.

We are also seeing renewed interest in what I would call the industry's 'senior citizens', mesenchymal stem cells (MSC). While these cells count among the first regulatory approvals in the cell therapy space, they never achieved lasting clinical or commercial success. However, through the knowledge and experience gained with CAR-Ts and genetic modification, as well as a much more robust understanding of differentiation protocols, novel MSC products could be on the horizon, addressing (auto-)immune indications more broadly. And speaking of autoimmune disorders—recent successes in treating systemic lupus erythematosus, idiopathic inflammatory myositis, and systemic sclerosis with a standard CD-19 CAR-T have renewed attention on the field and given an indication that many more patients might benefit from cellular immunotherapies.

Lastly, several of our authors discuss approaches to better direct cells to their target, to reduce toxicity, improve solid tumor penetration and be more efficacious overall. Novel LNPs and targeting antibodies are being considered, as well as combination therapies with adjuvants and checkpoint inhibitors. In my own Viewpoint article, I encourage you our readers to look to the medical device space, where catheters can be leveraged to deliver payload directly to the site of interest, thus addressing some of the off-target effect concerns and providing an opportunity to significantly reduce the required dose.

Overall, and in conclusion, we are approaching an era where our industry will have a solid repertoire of therapeutic modalities (autologous, allogeneic, *in vivo*, and combination therapies) and delivery approaches. This will require sustained investment not only into the development of therapeutics but also on the discovery side, where a fundamental understanding of cell and molecular biology will help underpin robust product development. We won't be bored anytime soon.

BIOGRAPHY

NINA BAUER is a trained neuroscientist. Her career quickly centered on the cell and gene therapy space, holding various technical and commercial roles at the Scottish Center for Regenerative Medicine and the Cell and Gene Therapy Catapult. She deepened her global commercial experience establishing Lonza's autologous cell therapy business and strategic positioning by incorporating the Cocoon™ technology with a vision for near-patient manufacturing, as well as leading commercial teams at MilliporeSigma/Merck KGaA focusing on the cell therapy product portfolio, gene therapy manufacturing services and CRISPR IP

licensing. Aside from large corporate and public sector leadership roles, Nina established her start-up and C-suite acumen as the Chief Commercial Officer for FloDesign Sonics, leading to the company's acquisition by MilliporeSigma, as well as a wide range of advisory board engagements. Nina currently serves as the Chief Business Officer of SmartCella, Stockholm, Sweden, a Swedish biotech developing therapeutic delivery approaches and pluripotent stem cell-based therapies.

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CELL & GENE THERAPY INSIGHTS

INTERVIEW

Following the data: lessons learned from the development of afami-cel

With Adaptimmune's afami-cel on the verge of making history as both the first approved engineered TCR-T cell therapy and the first cellular immunotherapy to be approved for a solid tumor indication, CSO **Jo Brewer** tells **David McCall** (Senior Editor, BioInsights) about the long and winding road to market for this ground-breaking product, and shares some insights gleaned along the way.

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Q What are you working on right now?

JB: Adaptimmune is focused on commercializing our first product for people with synovial sarcoma, which is called afami-cel. As CSO, my eyes are fixed on the longer-term future and filling our pipeline with whatever comes next. That means making the most of what we have learned from our current clinical success to develop new T cell products for different solid tumors to treat a wider population of patients.

At Adaptimmune, we have two T cell therapy platforms: engineered T cell receptors (TCRs), and T cell receptor fusion constructs (TRuCs), which is in essence an antibody binding domain stuck to a CD3 zeta chain. Having the two platforms allows us to look at a wide range of tumor targets. The engineered TCRs recognize internally expressed proteins that are then presented on the surface of the cell by human leuokocyte antigen (HLA). However, TRuCs work similarly to CARs by targeting membrane-bound proteins. They recognize surface targets like antibodies but use the physiological signaling machinery of the T cell, essentially bolting onto natural TCR signaling. Both of these approaches are fundamentally different from the CAR constructs where all the signaling domains are bolted onto a single molecule.

So, leveraging both platforms gives us a wide range of targets, and by combining these platforms with next-gen approaches, we can also seek to make T cells more potent and long-lasting.

Q Can you tell us more about progress with afami-cel—what else are you doing to lay the foundation for what the field hopes will be a landmark approval and subsequent successful commercialization?

JB: I have been in this field for well over 20 years, and so it is quite exciting that the first product of this type is now coming to market—hopefully, it is the first of many more from our pipeline. We submitted the BLA for afami-cel at the end of 2023, and it was accepted by the US FDA in January 2024. The FDA had given us a PDUFA date of August 4, 2024, and afami-cel was approved by the FDA last week, being sold as Tecelra® [1][2]. We have greatly benefited from our Managed Access Program (MAP) designation for afami-cel. This has enabled rapid review by the FDA and more frequent meetings with them throughout the process, which we have used to our advantage.

Synovial sarcoma is a rare disease, and patients are typically referred to specialist treatment centers rather than being treated in the community. We have been working with a lot of these specialized centers throughout our clinical trials and getting to know the individual physicians and their teams. We are planning to use the existing referral networks in the synovial sarcoma space. More specifically, we will start with six authorized treatment centers as we roll out and expand up to 30 sites across the USA. This will provide good coverage and minimize travel requirements for patients. This will be a small and focused launch, which is appropriate given the number of patients who are eligible for this treatment. (In order to get afami-cel, patients need to express two different biomarkers: they need to be HLA-A*02–positive, and their tumor needs to test positive for MAGE-A4).

It is also really important to have screening infrastructure in place, so that people can easily be tested for synovial sarcoma. The existing sites are familiar with the process as they have already been working with us through the clinical trials. Besides this, we have been working with patient advocacy groups to help us understand what patients need and how we can facilitate their access to afami-cel. In short, we are trying to make this as easy as possible for everyone involved to navigate—from the patients to the clinicians to the payers.

"...we did not set out to develop a synovial sarcoma drug, but that is where the first opportunity emerged."

Finally, we have scaled up our manufacturing capacity to support the launch of afami-cel. Commercial supply will continue from our Navy Yard facility in Philadelphia—the same facility and teams that supplied all of our clinical trials. We are trying to change as little as possible to ensure we continue to produce our best product.

Q How is the Adaptimmune R&D pipeline shaping up behind afami-cel—what is the strategic thinking/approach behind the next wave of product candidates in development?

JB: Our next cell therapy product is lete-cel, which is another TCR product for the treatment of synovial sarcoma as well as another type of soft tissue sarcoma, myxoid/round cell liposarcoma. With afami-cel, we expect to treat about 400 patients per annum, but together with lete-cel, the number could grow to 1,000, which would create a significant sarcoma franchise.

Lete-cel targets a different target biomarker—NY-ESO instead of MAGE-A4. However, both products are for HLA-A*02-positive patients who are treated at the same specialist centers by the same doctors. Therefore, our commercial footprint is exactly the same, allowing us to deliver both products seamlessly.

Afami-cel and lete-cel are both first-generation products, with a TCR introduced into a T cell. However, insights from those trials show us that in order to target other tumors, we need to go beyond just TCRs.

Q Can you go deeper on the clinical learnings you've gleaned from afami-cel and how these have led to a next-gen TCR-T cell therapy?

JB: The pilot study with afami-cel, originally designed as a basket trial, showed a few signals in other tumors as well. It was not aimed at synovial sarcoma in particular, but rather targeted any patient expressing HLA-A*02 and the MAGE-A4 antigen. We saw a response in patients with bladder, head and neck, and lung cancers, but these responses were not as deep and durable as those in synovial sarcoma. So, we did not set out to develop a synovial sarcoma drug, but that is where the first opportunity emerged. Looking at the data from the original patients, we see that not all T cells persisted. Afami-cel contains both killer CD8 and

"The more components of the immune system that are brought into play, the better the outcome might be for the patient."

helper CD4 T cells. However, over time, only CD8 cells were detectable in blood samples and CD4 cells were not present in the patients.

Recognizing the importance of CD4 cells in CAR therapies has led to the development of our next-generation uza-cel product. This drug uses the same TCR as afami-cel, targeting the MAGE-A4 biomarker, with a CD8 alpha co-receptor added as well. It effectively turns CD4 cells into a hybrid CD4/CD8 phenotype, stabilizing the binding activity of the TCR. As a result, these cells exhibit greater potency, proliferation, persistence, and anti-tumor activity in a broad range of solid cancers as well, which was not the case with the previous generations. These cells gain extra killing ability while maintaining their helper function and cytokine support, which is also important in allowing the CD8 T cells to do their job.

This iterative platform development process involves applying clinical learnings back into research. We are currently exploring this approach in ovarian cancer through the SURPASS-3 trial.

Q Adaptimmune is pursuing both monotherapy and combination therapy pathways with uza-cel—can you tell us more about the clinical development strategies for each, and share your thoughts more broadly on how the mono/combination therapy picture will continue to evolve in the immuno-oncology space?

JB: The success of the immune checkpoint inhibitor antibodies (CPIs) is legendary, and there are lots of reasons to combine them with a T cell product. Firstly, CPIs have not worked for ovarian cancer, one of the reasons being the fact that it is a cold tumor that T cells struggle to recognize. However, from our uza-cel data, we know that our engineered T cells can infiltrate ovarian tumors and also recruit other naturally occurring T cells, as we see both types present in patient tumor samples after treatment. We are hoping that the checkpoint blockade could benefit both the added TCR-T cells and the patient's natural T cells. The more components of the immune system that are brought into play, the better the outcome might be for the patient. Our trial includes two approaches—one using T cell therapy alone, and the other with additional checkpoint blockade to see if the combination improves the response rate or duration.

Q What particularly excites you in terms of cellular immunotherapy technologies that are just on or over the horizon?

JB: I think it is fantastic to be part of a field where autologous T cell therapies are already showing efficacy. They work in hematological malignancies, and we have demonstrated efficacy in solid tumors as well. Looking ahead, we want to make these therapies cheaper and more accessible to more patients. In order to achieve that, it will likely involve transitioning to allogeneic therapies and exploring *in vivo* delivery methods—both of these areas are very exciting.

Whilst we are focused on leveraging the existing knowledge in autologous therapies, which will continue to be a significant part of our business in the coming years, we are looking into allogeneic approaches as well. We are working on a stem cell-derived platform where we aim to replicate the success of autologous therapies using T cells derived from stem cells. The goal is to develop an off-the-shelf product that bypasses some of the supply chain complexities associated with autologous therapies.

If the *in vivo* delivery approach is effective in delivering the vector straight to the cells without culturing them outside the patient, it would be beneficial from a business perspective. However, this approach is currently unproven in the clinic.

Both allogeneic therapies and *in vivo* delivery are areas of significant current innovation. In the future, I envision that all three approaches—autologous, allogeneic, and *in vivo* delivery—will be used to gain deeper insights into how these therapies work in different tumors.

I think there is a real element of fashion in investing in this space. Allogeneic therapies have lost some popularity because they pose a greater scientific challenge than people thought, and there is no quick fix. Similarly, I do not think *in vivo* delivery is going to be a quick fix, despite being an exciting new area of research. I do think that both approaches will eventually work, but addressing the scientific and practical challenges will require long-term commitment. Perhaps in contrast to how these therapies are being pitched, it is not a 5-year investment to achieve success.

The science behind these advancements is great, but there are many challenges that take time to navigate. For example, lete-cel is now our second product, but it was originally our initial focus back in 2008. We have learned a lot since then, including considerations regarding lymphodepletion, target density, and manufacturing. We then partnered with GlaxoSmithKline, who took lete-cel in and changed the manufacturing process, which caused delays. Meanwhile, we brought forward afami-cel using the earlier manufacturing approach. Both therapies happened to be effective against synovial sarcoma, which was a bit of serendipity. Subsequently, GlaxoSmithKline made the strategic decision to exit the cell therapy field and gave the lete-cel product back to us.

Due to its different manufacturing platform, lete-cel required the repeating of trials, and resulted in two separate processes for our products. Lete-cel has probably taken 6 years longer to get to the same stage as afami-cel because we were able to shortcut many processes for afami-cel thanks to lessons we learned with lete-cel.

Q It has been a long road that has led to Adaptimmune standing on the brink of such an important breakthrough approval for the cell and gene therapy field—what key learnings would you derive from the experience that can help early-stage developers?

JB: I think one of the key things is having the ability to pivot and follow the data. As I mentioned, initially, we were hopeful of achieving long-term success with our first product, but that was not to be the case. Focusing on synovial sarcoma wasn't the main aim at first, but it is amazing how we developed something that could be game-changing in this particular area. In this industry, you have to pursue opportunities when they arise. Finding your niche is crucial and oftentimes, it might not be what you originally anticipated. The competition to enroll patients in trials is fierce, especially in areas where there are a lot of other treatment options, such as CPIs. Therefore, being open to pivoting and changing based on what the data is telling you is important.

It is also crucial to find good investors who understand the field, and who are committed to long-term collaboration rather than seeking a quick turnaround. While we cannot always choose our investors and they are often very selective, having the right ones can help navigate the difficult times that often arise in science. Things may go wrong, but you have to stay focused and dogmatic. If you believe in your vision, you will find ways to push through and generate the data that takes you to the next stage.

In summary, there are always challenges to overcome. It is all about finding ways to get to the next stage and being able to recognize opportunities when they arise rather than overlooking them—that is a valuable skill to have for people in the early stages of therapy development.

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BIOGRAPHY

JO BREWER is the Chief Scientific Officer at Adaptimmune, Oxford, UK generating innovative cell therapies for people with cancer. Jo has more than 20 years of experience in immunotherapy research across the breadth of discovery and translational science, developing both autologous and allogeneic T cell platforms. She was one of the founding scientists at Adaptimmune, who built multiple research teams developing the first gen TCR-T cell therapies afami-cel and lete-cel for sarcoma. She is co-inventor of the next-gen product

uza-cel, currently in Phase 2 for ovarian cancer. Prior to joining Adaptimmune in 2009, Jo held positions at Avidex and Medigene. She holds an MSc in Natural Sciences and a PhD in Cellular Signaling, both from the University of Cambridge, Cambridge, UK.

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CELL & GENE THERAPY INSIGHTS

INNOVATION IN CELLULAR IMMUNOTHERAPY:
HOW TO REACH MORE PATIENTS?

SPOTLIGHT

EXPERT INSIGHT

Patient-specific factors that could influence gene therapy outcomes in sickle cell disease

Abdulrahman Alsultan and Jaap Jan Boelens

Sickle cell disease (SCD) is a chronic, debilitating inherited blood disorder that is considered one of the most common inherited disorders worldwide. Despite its description early in the last century, there is still no curative therapy apart from hematopoietic cell transplant (HCT), which requires the availability of suitable donors. The recent approval of gene therapy in SCD provides new treatment options for individuals with SCD. However, it is still far from ideal, as it requires (like HCT) myeloablative conditioning for the engraftment of genetically modified cells and may not fully cure the disease, but rather ameliorate its severity. This article will discuss potential host factors that may impact the overall success of gene therapy in SCD to be considered in future studies.

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Sickle cell disease (SCD) is a chronic, debilitating inherited blood disorder that is considered one of the most common inherited disorders worldwide [1]. Despite its description early in the last century, there is still no curative therapy apart from hematopoietic cell transplant (HCT), which requires the availability of suitable donors [2]. The recent approval of gene therapy in SCD provides new treatment options for individuals with SCD. However, it is still far from ideal, as it requires (like HCT) myeloablative conditioning for the engraftment of genetically modified cells. And even with full engraftment of genetically modified cells, it may not fully cure the disease, but rather ameliorate its

severity [3,4]. Less intense conditioning will give worse correction of the disease because of lower engraftment of genetically modified cells. Protocols on how to navigate HCT in SCD have been well developed, leading to a high cure rate that exceeds 90% [5,6]. In contrast, gene therapy uses the patient's own cells rather than healthy donor cells, introducing various host factors that may influence different phases of gene therapy. These phases include the ability to mobilize SCD patients efficiently, manufacture high-quality products with optimal cell dose, ensure the persistence of the graft (over a time period of decades), and achieve goals in preventing sickling and hemolysis. Collecting real-world data is essential and may help identify key factors associated with outcomes. This article will discuss potential host factors that may impact the overall success of gene therapy in SCD to be considered in future studies (Table 1).

AGE

Gene therapy in SCD is approved in patients ≥12 years of age, but given the small sample size in the reported clinical trials, the impact of age may manifest when assessed in real-world data [3,4]. The severity of SCD phenotype increases with age, and older patients often present with higher rates of comorbidities, such as cardiopulmonary complications and chronic renal insufficiency, which could make them less tolerant of myeloablative conditioning [7]. Aging was shown to negatively impact the ability to mobilize hematopoietic stem cells (HSCs) in SCD patients [8]. In addition, age-related changes in HSCs, such as increased genomic instability and diminished regenerative capacity, as well as age-related alterations in the bone marrow niche, may impact the quality and persistence of genetically modified HSCs [9]. Younger patients may fare particularly well, similar to HCT [5]. Thus, standardized collection of real-world data is essential to better understand the impact of age.

SEX

Variation in the SCD phenotype has been reported between males and females. Females with SCD live longer and have, on average, higher HbF levels [10,11]. While no sex-based outcome difference has been reported in HCT or gene therapy for SCD, it is worth studying in larger cohorts whether such differences exist particularly in gene therapy aiming to augment gamma-globin gene expression.

GENETIC MODIFIERS

Despite SCD being a monogenic disorder, different genetic modifiers may alter the severity of SCD, HbF level, and intensity of hemolysis. Collecting information in a standardized manner, in real-world cohorts will also help us to better understand what impact on outcomes is going to be.

HbF modifiers

- *HBB* haplotypes: the severity of SCD and level of HbF correlates with *HBB* haplotypes, with the severe disease and lowest HbF present in Bantu and Benin haplotypes and less severe SCD and highest HbF present in Arab-Indian haplotype. Clinical trials of gene therapy in SCD included only patients with low HbF (<10%), so it is not clear how *HBB* haplotypes will impact the efficacy, particularly if HbF augmentation is the goal. In addition, hyposplenism/autosplenecomty is common in African-origin haplotypes, but preserved spleen and splenomegaly are frequent in the Arab-Indian haplotype. While this is unlikely to impact HSCs mobilization using plerixafor, as shown in β-thalassemia, further studies are needed as Arab-Indian haplotype patients were not included in reported trials [7,12,13]. In addition, it is important to understand the impact of splenomegaly on the risk of primary and secondary graft failures post-gene therapy [14].

TABLE 1

Patient-specific factors that could influence gene therapy outcomes in sickle cell disease: considerations for future studies.

Factor	Description
Age	Adults vs adolescents/children
Sex	Male vs female
Genetic modifiers	▶ HbF modifiers: ▶ HBB haplotypes (African origin haplotypes vs Arab-Indian haplotype) ▶ Trans-acting genetic modifiers: variants in activators or repressors of gamma-globin expression ▶ Co-inheritance of alpha thalassemia ▶ G6PD deficiency
Iron overload	Impact of excess iron on different phases of gene therapy
Concomitant medications	Determine the optimal duration of hold of hydroxyurea and other SCD-modifying agents
Malnutrition	Impact of optimizing nutritional status on gene therapy outcome
Psychosocial factors	Influence of psychosocial factors on gene therapy outcome

- Trans-acting genetic factors: the mechanism of the globin switch from fetal hemoglobin (HbF) to adult hemoglobin (HbA) is complex, involving multiple trans-acting genetic factors such as BCL11A, ZBTB7A/LRF, NF-Y, among others. Given the expanding list of genes involved, it remains unclear how polymorphisms in activators or repressors of gamma-globin expression will affect HbF levels in patients with SCD following gene therapy, especially when targeting only one specific gene, such as *BCL11A* [15,16].

Co-inheritance of alpha thalassemia

Co-inheritance of alpha thalassemia is present in one-third to half of SCD patients, is associated with less hemolysis and higher baseline hemoglobin, and could impact the severity of SCD [7]. The Lovo-cel product considers alpha thalassemia a limitation due to reports of anemia/myelodysplastic syndrome with deletional alpha thalassemia [17]. It is important to also test for non-deletional alpha thalassemia in patients from certain geographic areas, which may not be routinely tested in some reference laboratories [18].

G6PD deficiency

G6PD deficiency is common among SCD patients with no strong link between G6PD deficiency and SCD severity [19]. We did not observe differences in outcomes among SCD patients with G6PD deficiency who underwent HCT, nor did we see differences if donors had G6PD deficiency, provided that triggers of hemolysis were avoided [Unpublished Data]. It will be interesting to investigate whether G6PD deficiency alters the response to oxidative stress during genetic manipulation of HSCs, and potentially impacts the proliferation and quality of HSCs.

IRON OVERLOAD

Although physicians typically aim to minimize liver and cardiac toxicities by managing iron overload with iron chelation before administering a myeloablative conditioning regimen, this management might not be prioritized before HSC mobilization for gene therapy. Excess iron can impair the proliferation capacity of HSCs, increase reactive oxygen species levels, and induce apoptosis [21]. Consequently, iron overload could potentially affect the efficiency of HSC mobilization, the

quality of genetically modified HSCs, and engraftment. Therefore, the impact of iron overload on different phases of gene therapy needs to be thoroughly studied.

CONCOMITANT MEDICATIONS

It is recommended to stop hydroxyurea and other SCD-modifying agents (e.g., voxelotor and crizanlizumab) before mobilization to ensure optimal cell dose collection. When these agents are held, it is necessary to initiate exchange transfusion (to reduce risk on crisis, and keep SCD as controlled as possible). The reported cessation duration of hydroxyurea ranges from a few weeks to a few months; however, a 2-month hold is recommended for currently approved gene therapy [22–24]. One study showed a positive correlation between the number of days hydroxyurea was held and mobilized cell dose using plerixafor in SCD [8]. The optimal duration of hold is yet to be defined and may differ among patients on optimal doses, non-compliant patients, and those with dose-limiting toxicities such as neutropenia or thrombocytopenia. However, holding hydroxyurea can exacerbate SCD severity, challenging the length of the hold.

MALNUTRITION

Malnutrition is common among patients with SCD, including deficiencies in essential vitamins such as vitamin D, folate, vitamin B12, and some minerals. Malnutrition can exacerbate the severity of SCD [25]. Optimizing nutritional status prior to HSCs mobilization may enhance the efficiency of HSCs mobilization, improve the quality of genetically modified cells, and ultimately increase tolerance to myeloablative conditioning.

PSYCHOSOCIAL FACTORS

Psychosocial factors such as depression, anxiety, fatigue, and financial hardship are common among SCD patients [26]. It is important to address these factors to optimize the mental well-being of SCD patients undergoing gene therapy [27]. It is also essential to provide realistic expectations about the outcomes of current gene therapy in SCD; patients may experience frustration if their expectations for a cure are not met, which can negatively impact their overall mental health [28].

CONCLUSION

Gene therapy for SCD is a novel, highly desirable new development, and having access to products approved by the US FDA, the EMA, and other authorities is revolutionary. However, we must acknowledge that the data is still young and developing; the median follow-up of the two currently approved gene therapy products is still only in the regio of 2 years (while HCT follow-up is in the decades, albeit that HCT is associated with more complications). To better understand what the long-term outcomes will be, standardized real-world data collection and analyses are of utmost importance. This will ultimately lead to better patient selection and outcomes.

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CELL & GENE THERAPY INSIGHTS

INNOVATION IN CELLULAR IMMUNOTHERAPY: HOW TO REACH MORE PATIENTS?

SPOTLIGHT

INNOVATOR INSIGHT

Enhancing CAR-T cell generation: optimizing non-viral engineering of resting T cells for improved cancer immunotherapy

Akshaya Chandrasekaran

Resting T cells are a highly desirable starting material for CAR-T cell production due to their persistence, capacity for self-renewal, and genomic stability. This article introduces a system that could be used to optimize the generation of CAR-T cells from resting T cells as part of a clinically relevant CAR-T cell therapy manufacturing workflow that helps ensure safety, efficacy, and scalability. The system, specifically designed for therapeutic cell engineering, harnesses a lower conductivity buffer to help improve transfection efficiency and cell viability with non-viral gene delivery.

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INTRODUCTION TO THE APPLICATION OF CLOSED MODULAR SYSTEMS IN THE CAR-T CELL THERAPY MANUFACTURING WORKFLOW

CAR-T cell therapy has been revolutionary in producing durable and effective clinical responses in patients. However, the traditional manufacturing process is complex and labor-intensive, involving several open unit operations that can impact regulatory compliance. Fortunately, there is now a series of closed instruments available that can help minimize the errors and contamination associated with open process steps in cell therapy manufacturing. These modular instruments can operate either independently, or collectively as an integrated, closed, automated workflow.

Figure 1 represents the traditional CAR-T workflow, highlighting available closed modular instruments and products available from Thermo Fisher Scientific at each stage. All of the solutions depicted in red can be utilized from process optimization and development through to commercial manufacturing, with full regulatory documentation and support available. This article will focus on a non-viral cell engineering workflow that utilizes the Gibco™ CTS™ Rotea™ Counterflow Centrifugation System, and the Gibco™ CTS™ Xenon™ Electroporation System for the large-volume transfection of resting T cells.

EDITING NON-ACTIVATED T CELLS IN CAR-T THERAPY MANUFACTURING

It is important to the success of non-viral cell engineering to establish an end-to-end workflow using a non-activated (resting) T cell starting population, due to the advantages

FIGURE 1

Traditional CAR-T workflow with key instruments and products discussed in this article highlighted in red.

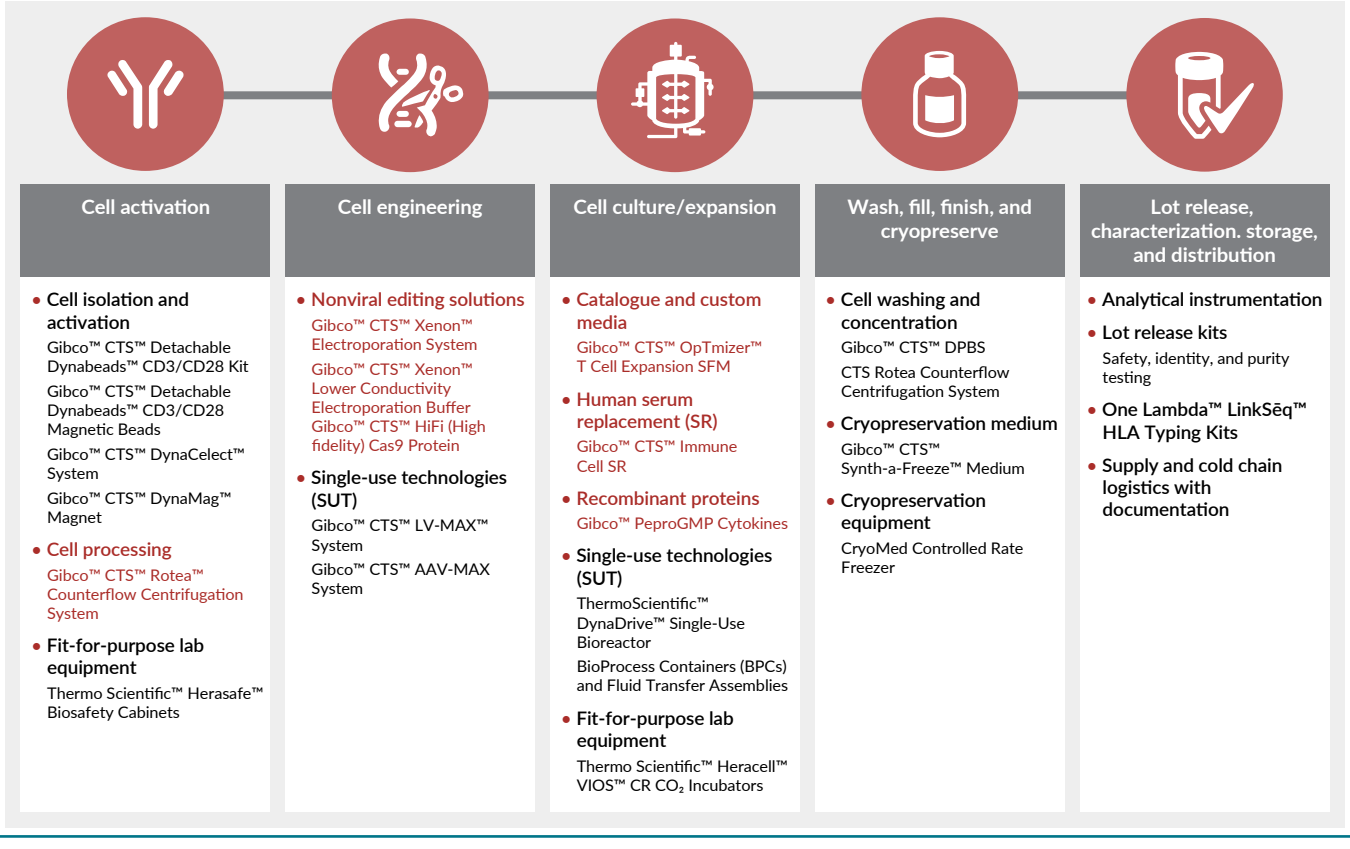


FIGURE 2

Naïve T cells (T_N) and the four subsequent subtypes (T memory stem cells [T_{SCM}], central memory T cells [T_{CM}], effector memory T cells [T_{EM}], and terminal effector T cells [T_{TE}]) identified by their expression of CD45RA and CD62L.

	T_N	T_{SCM}	T_{CM}	T_{EM}	T_{TE}
CD45RA	+	+	–	–	+
CD62L	+	+	+	–	–
CD45RO	–	–	+	+	–
CCR7	+	+	+	–	–
CD28	+	+	+	±	–
CD27	+	+	+	±	–
IL-7Rα	+	+	+	±	–
CXCR3	–	+	+	–	–
CD95	–	+	+	+	+
CD11a	–	+	+	+	+
IL-2Rβ	–	+	+	+	+
CD58	–	+	+	+	+
CD57	–	–	–	±	+

Stemness
Proliferation potential
Lymphoid homing
Antigen independence
Lipid metabolism
Low delta psi(m)

Senescence
Cytotoxicity
Tissue tropism
Antigen addiction
Glycolytic mechanism
Oxidative stress

conferred by the naïve T cell (T_N) population. T_N are those cells that have yet to encounter an antigen and remain unstimulated and quiescent in nature. Upon antigen stimulation, T_N can progressively differentiate into four subsets of memory T cells: T memory stem cells (T_{SCM}), central memory T cells (T_{CM}), effector memory T cells (TEM), and terminal effector T cells (T_{TE}). These distinct cell populations can be identified based on the expression of CD45RA and CD62L, as shown in Figure 2.

Among the T cell subsets, T_N and T_{SCM} are the least differentiated and represent the long-lived T cell memory subset. These cells possess a strong capacity for self-renewal and multipotent differentiation into other memory cell subsets. However, the relative scarcity of T_{SCM} in circulation necessitates *ex vivo* expansion protocols to enable their effective use in clinical applications.

A review of early CAR-T trials involving patients with reduced remission rates showed higher levels of T_N and T_{SCM} in the starting material. This undifferentiated, intrinsically less activated cell state means that in cases of tumor relapse, these cells are exceptional in their ability to expand and differentiate into effector T cell types. This, in turn, leads to reduced T cell exhaustion and an enhanced, more persistent anti-tumor effect when used in adaptive immunotherapy.

Recent studies have demonstrated that CAR-T cell therapies derived from T_N or T_{SCM} populations exhibit improved tumor control *in vivo* while also reducing the risk of cytokine release syndrome compared to conventional CAR-T therapies. Furthermore, the

cells can be cultured in the presence of specific cytokines or modulated through specific cell signaling pathways in order to produce a desirable stem-like phenotype [1,2].

A longer duration of culture *ex vivo* is associated with increased T cell exhaustion. Therefore, it is also crucial to shorten the T cell manufacturing workflow. Novel manufacturing protocols that can deliver this combination of improved T cell phenotypes and a shorter production timeframe are key to enabling the allogeneic cell therapy field in particular.

In a CAR-T manufacturing workflow utilizing non-activated or resting T cells, the T cells are not activated before gene editing. This means that the resultant CAR-T product can then be infused to allow for continuous antigenic stimulation and activation *in vivo*, where the cells will retain their replicative capacity to maintain function. Thus, non-activated T cells are highly desirable as a starting material for their ability to allow reduced workflow time, which is critical for both enabling decentralized (including point-of-care) manufacturing and reducing cost of goods (COG) [1–4].

Editing non-activated T cells also confers the advantage of mitigated chromosomal loss and a reduced frequency of indels of up to 13% compared to editing activated T cells, as reported in recent studies [5]. It has been observed that the site-specific CRISPR/Cas9-mediated genome editing of T cells resulted in unintended but targeted and persistent chromosome loss [5]. Although this chromosomal loss is a universal consequence, novel workflows that enable the editing of non-activated T cells can minimize its overall occurrence and impact.

AN END-TO-END NON-VIRAL CELL ENGINEERING WORKFLOW

There are two main strategies employed for engineering primary T cells: viral transduction and non-viral transfection. The limitations in using viral vectors to produce T cell therapies, including cost and safety, necessitate the development of non-viral alternatives that allow for sophisticated and targeted engineering. The most prevalent non-viral method employed in this field is electroporation.

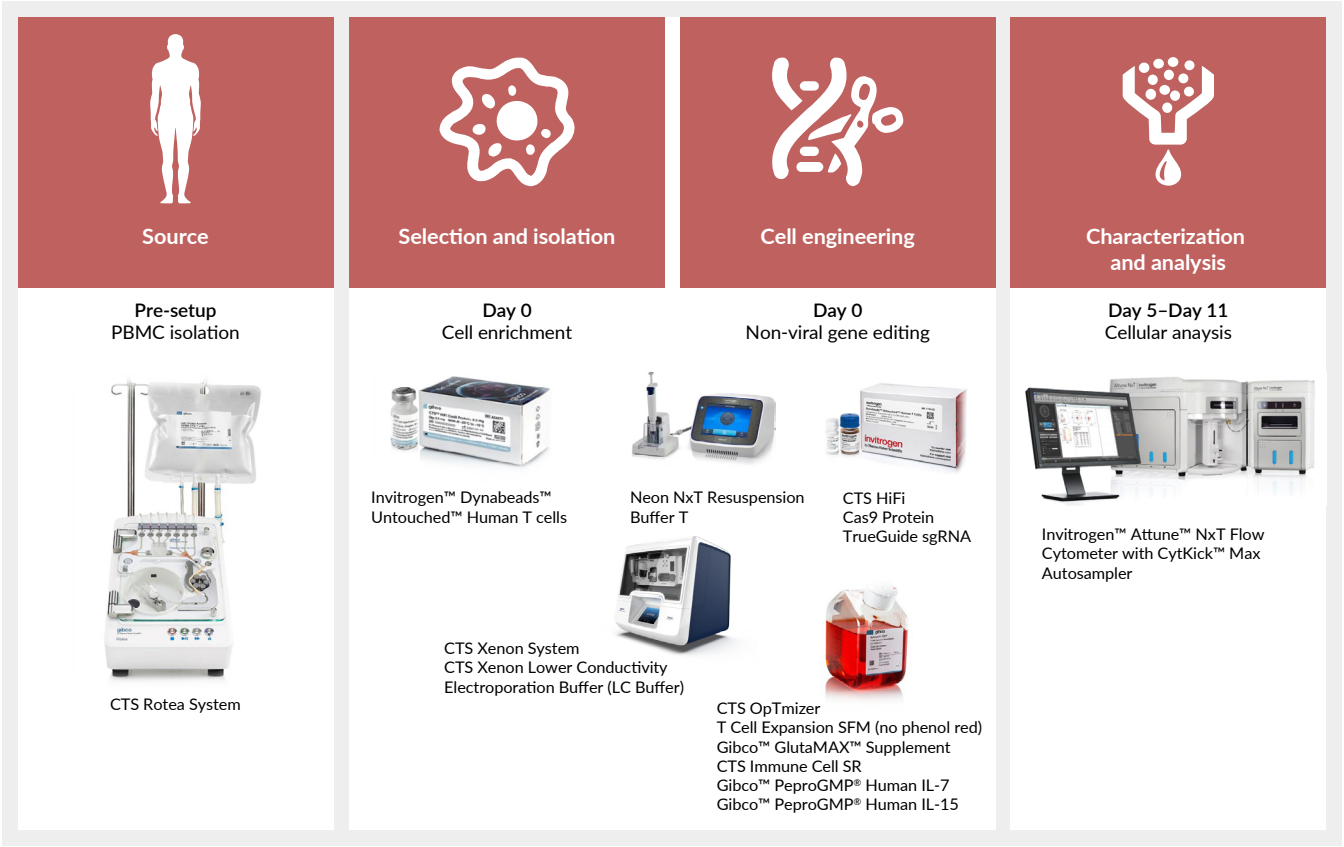
Thermo Fisher's end-to-end non-viral cell engineering workflow for non-activated T cells, which is depicted in **Figure 3**, begins with the processing of fresh healthy donor leukopaks on the CTS Rotea Counterflow Centrifugation System to isolate peripheral blood mononuclear cells (PBMCs). The CTS Rotea System allows for several flexible operations including cell separation based on size, wash, concentration, buffer exchange, and formulation. Once the PBMCs are isolated, pan T cells are enriched and resuspended in the Neon NxT resuspension Buffer T or Gibco™ CTS™ Xenon™ Lower Conductivity Electroporation (CTS Xenon LCE) Buffer before electroporation on either the Invitrogen™ Neon™ NxT or CTS Xenon electroporation systems.

The T cells are then cultured in Gibco™ CTS™ OpTmizer™ Serum-Free Media (SFM), no phenol red, supplemented with several additives including Gibco™ PeproGMP™ cytokines. The cells are cultured up to day 11 post-electroporation, and a cellular analysis is then performed on the Invitrogen™ Attune™ NxT Flow Cytometer on days 5 and 11 post-electroporation to quantify target gene knockout (KO) and transgene knock-in (KI).

Both the Neon NxT and CTS Xenon electroporation systems help ensure reliable delivery of DNA/RNA, proteins, and other molecules into cells while maintaining cell viability and recovery. These systems offer user-programmable settings, allowing for the creation and optimization of electroporation protocols for various cell types and payloads, from early optimization (Neon NxT system) through process development and commercial GMP manufacturing (CTS Xenon system). The Neon NxT can be used for optimization of non-viral editing. However, the CTS Xenon system can be paired with other

FIGURE 3

End-to-end non-viral cell engineering workflow of non-activated (resting) T cells.



instruments to form a closed, integrated, GMP-compliant workflow.

The CTS Xenon LCE Buffer enhances electroporation performance in cell therapy development and clinical manufacturing workflows by expanding the system's utility for transfecting various cell types, especially non-dividing or non-proliferative cells. Performance is improved as the buffer allows for higher energy settings, specifically benefiting non-proliferative cells. The low conductivity formulation enables a greater range of variations and combinations in the electroporation process including voltage, pulse width, and pulse number. It can further support applications involving heat-sensitive cells or transfections performed at lower cell density by minimizing heat generation during electroporation.

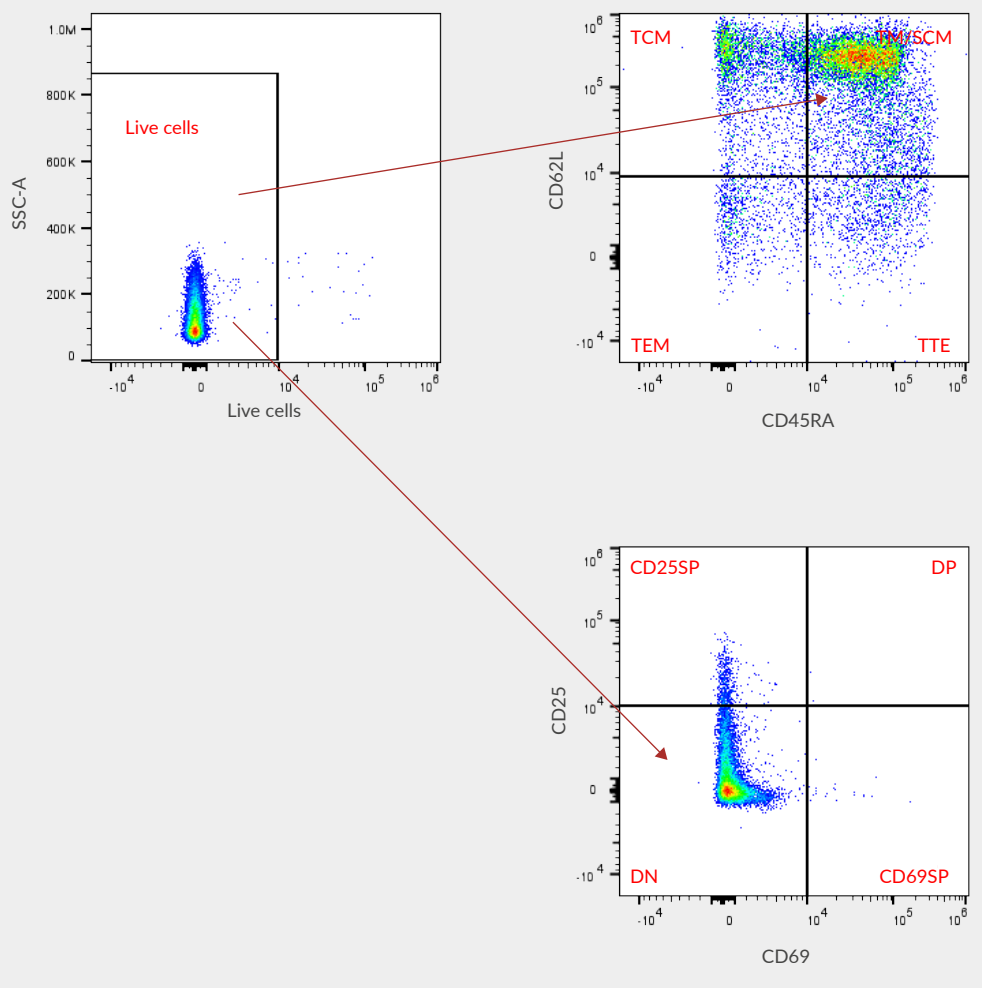
The non-activated T cell workflow demonstrated here aims to address three key challenges associated with traditional CAR-T manufacturing. The first issue is reducing manufacturing times and costs, which can be addressed through having the ability to complete a non-activated T cell workflow in 1 day. Secondly, reducing off-target effects can be accomplished by using the CTS HiFi Cas9 protein, and thirdly, successful transfection of a non-activated cell is enabled through use of the CTS Xenon LCE Buffer.

T CELL ENRICHMENT, CELL CULTURE, AND ENGINEERING WORKFLOW

The non-activated T cell gene editing workflow begins with solutions for T cell enrichment, cell culture, and engineering. As with the end-to-end non-viral cell engineering workflow, PBMCs are isolated from healthy donor apheresis and enriched for pan T cells.

FIGURE 4

Gating strategy of CD45RA and CD62L double-positive cells as parent gates following derivation of live T cell populations to assess transfection or KO/KI efficiency.



As previously mentioned, these processes are designed to enable transfection and editing of the non-dividing, non-activated T cell population.

In a study to analyze the efficiency of the workflow and specifically, the editing of the naive T_{SCM} population, CD45RA and CD62L double-positive cells were gated. The results are shown in Figure 4. These served as the parent gates for assessment of transfection or KO/KI efficiency. Maintaining the non-activated phenotype was critical for downstream functionality, and this workflow helped to ensure that the process did not induce activation through monitoring of the expression of CD69 and CD25—the early and late activation markers—before and after non-viral modifications.

A comparison of the expression of CD3 and T cell subsets, including CD45RA and CD62L double-positive naive T cells, was conducted pre- and post-pan T cell isolation, with the results shown in Figure 5. The percentage of live cells remained unaffected between pre- and post-isolation. The two graphs illustrate the effectiveness of using the Invitrogen™ Dynabeads™ Untouched™ Human Cells kit. PBMCs were enriched for CD3-positive pan T cells by negative isolation with this kit, which depletes B cells, NK cells, monocytes, platelets, dendritic cells, granulocytes, and erythrocytes.

Representative flow plots for pre-isolation and post-isolation cells are provided in Figure 6, demonstrating that pan T cell enrichment was achieved while maintaining viability and surface marker expression pre- and post-isolation.

The next step in the workflow saw the post-pan T cell enrichment cells resuspended in the CTS Xenon LCE buffer. Electroporation was performed using either the Neon NxT or the CTS Xenon electroporation systems. CTS HiFi Cas9 protein was used in combination with the TrueGuide™ Synthetic guide (g) RNA to form the Cas9 RNP for gene editing.

The CTS OpTmizer SFM with CTS Immune Cell SR maintained stemness and phenotype similar to T cells cultured in conventional serum. The medium was supplemented with PeproGMP IL-7 and IL-15, which have been demonstrated to significantly improve cell survival without inducing proliferation or activation. The cell culture medium was refreshed every 3 days throughout the process. Surface marker analysis was performed using the Attune NxT Flow Cytometer on days 5 and 11 post-electroporation to quantify target gene KO and transgene KI.

TRANSIENT GENE EXPRESSION: mRNA DELIVERY

The non-viral engineering workflow can be employed to deliver a variety of payloads to non-activated T cells. Transient transgene delivery is achieved using green fluorescent protein (GFP) mRNA and plasmid-based payloads. Figure 7 gives a detailed overview of the specific experimental parameters and conditions utilized for a study of GFP mRNA delivery.

Resting T cells were electroporated with GFP mRNA at a concentration of 0.75 µg/1×10^6 cells using the Xenon electroporation system. The LCE buffer was employed to enable a higher energy setting. This involved the application of a higher voltage in combination with a longer pulse width—specifically, a single pulse of 2,200 V with a 20-millisecond pulse width. The GFP transfection efficiency was measured 24 hours post-electroporation within the naive CD45RA and CD62L expressing T cell population. The representative flow plots seen in Figure 8 depict percent GFP expression in control samples electroporated without the payload and in samples electroporated with the payload across two different donors.

The graphs in Figure 9 include appropriate controls that were either untreated (-/-) or subject to only electroporation (+/-) or only mRNA incubation (-/+). Successful transfection of GFP mRNA was demonstrated in the non-activated T cell population, with the maintenance of the percent live cells. GFP expression was donor-dependent, however, transient transfection of mRNA was made possible using the Xenon LCE buffer.

FIGURE 5

Comparison of CD3 and T cell subsets (T_{SCM}, T_{TE}, T_{CM}, T_{EM}) expression pre- and post-pan T cell isolation.

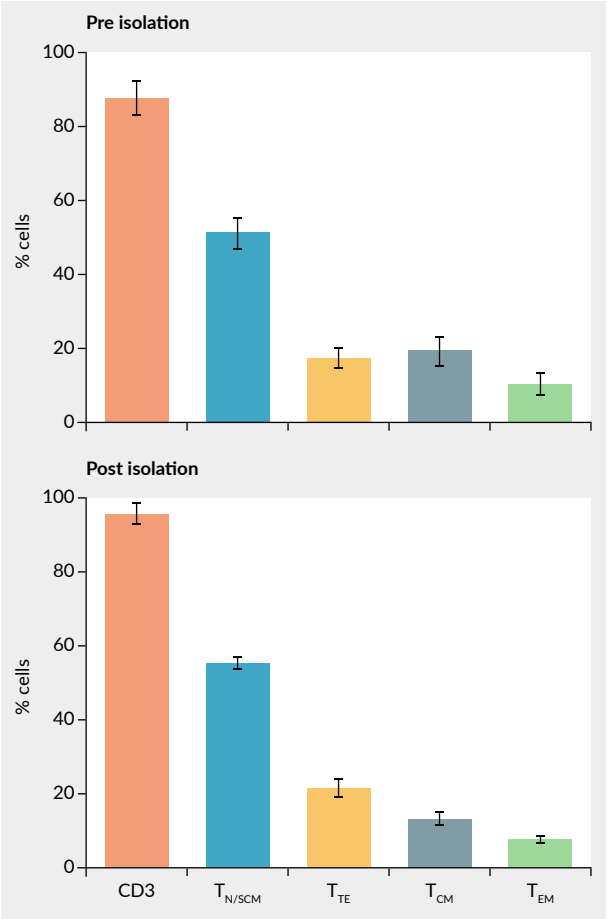


FIGURE 6

Flow plot comparison of live cells, CD3, and CD45RA⁺/CD62L⁺ populations pre- and post-isolation.

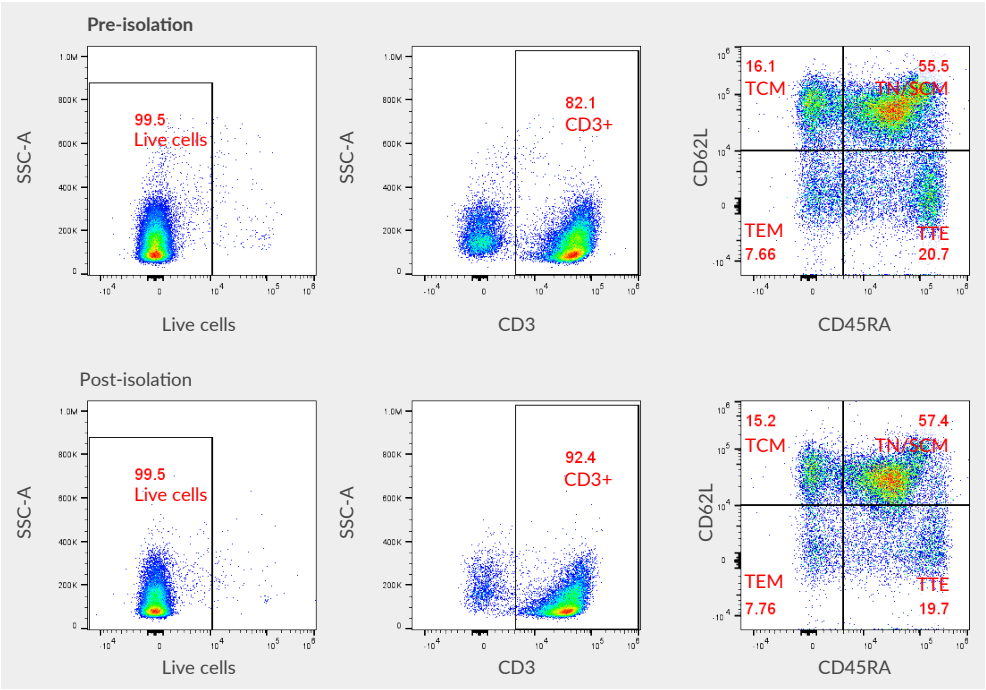


FIGURE 7

Experimental parameters and conditions employed for GFP mRNA delivery.

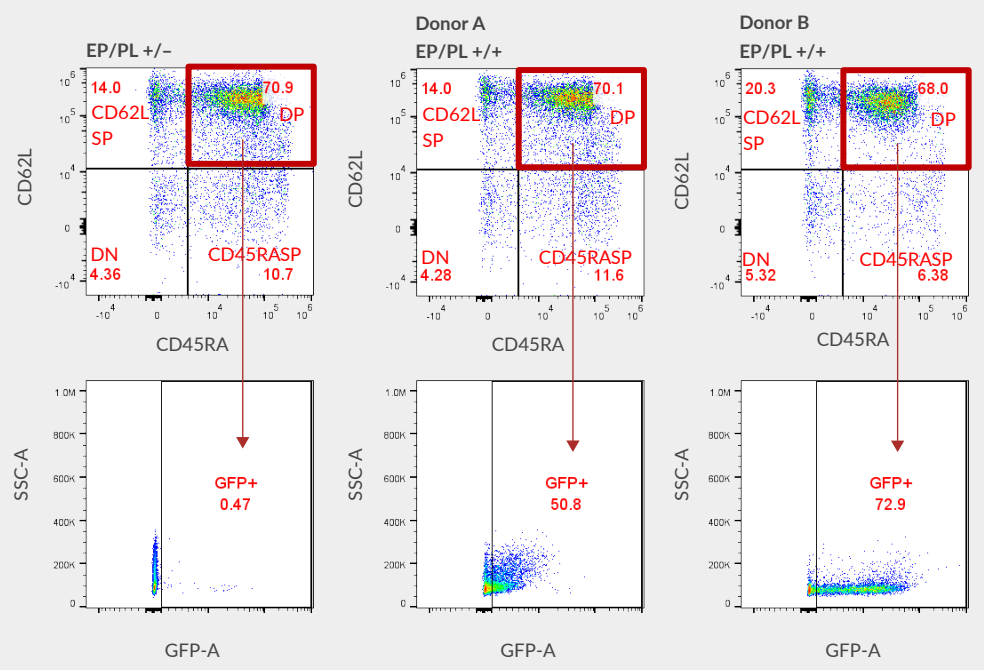
Experimental details	
Cell source and isolation	PBMCs were isolated from fresh leukapheresis products from two healthy donors using the CTS Rotea system; Pan T cells were isolated from PBMCs using Dynabeads Untouched Human T Cells Kit
Samples	N=2 donors
Concentration of cells during electroporation and electroporation scale	CTS Xenon Electroporation System: 50×10^6 cells in 1 mL SingleShot
Payload	eGFP mRNA (0.75 µg/1 × 10^6 cells)
Buffers	CTS Xenon Lower Conductivity Electroporation Buffer
Electroporation protocols	CTS Xenon Electroporation System (2200 V, 20 ms, 1 pulse)
Culture vessel and post-electroporation seeding density	24-well plates; 1 × 10^6 cells/mL

GFP mRNA delivery using the LCE buffer did not impact the naivety or stemness of the starting population, as evidenced by the representative CD45RA and CD62L plots and graphical representation in Figure 10. Increasing fractions of T_{CM}, T_{EM}, and T_{TE} indicate T cell differentiation wherein the LCE buffer enabled the preservation of the naive phenotype, which is crucial to downstream functionality.

The LCE buffer also induced neither early nor late activation markers, as was observed in the population post-electroporation (Figure 11). In the left panel, results are shown

FIGURE 8

GFP transfection efficiency post-electroporation of the control sample, donor A, and donor B with (EP/PL +/+) or without payload (EP/PL +/-) after 24 hours within the naïve CD45RA and CD62L expressing T cell population.



for samples that were electroporated in the absence of the payload versus the two donor samples that received the payload via electroporation. Figure 11 also shows that the buffer enabled transfection while maintaining phenotype and did not induce spontaneous activation or proliferation of T cells.

TRANSIENT GENE EXPRESSION: PLASMID DELIVERY

In a GFP plasmid delivery study, different cell densities at the time of electroporation were tested at a plasmid concentration of 5 µg/1×10^6 cells. The CTS Xenon LCE Buffer was utilized to perform electroporation on the Xenon electroporation system with higher energy settings of 2,200 V for a single pulse with a 20-millisecond pulse width. Further experimental parameters and conditions can be seen in Figure 12.

72 hours post-electroporation, the GFP transfection efficiency was measured within the naive CD45RA and CD62L expressing T cell population. The representative flow cytometry plots in Figure 13 depict GFP plasmid expression in control cells versus cells electroporated with the payload across both donors. Two different cell densities were tested at the time of electroporation: 25×10^6 cells and 50×10^6 cells per reaction in the Xenon SingleShot Electroporation Chamber.

The maintenance of the live cell population percentage across both cell densities demonstrated the protective effects of the lower conductivity formulation, especially when working with slightly lower cell densities during electroporation. In terms of GFP transfection efficiency, a donor-dependent effect was observed, with a maximum transfection efficiency of 40% in the non-activated T cells.

GENE EDITING: KNOCKOUT AND KNOCK-IN

After optimizing various parameters and conducting transient transfection experiments,

the determined configurations were implemented in a hyphenate non-viral cell therapy manufacturing process. This process involved initially knocking out β2M in two distinct T cell donors. To showcase the scalability of the tested conditions, cells were electroporated using the Neon NxT and CTS Xenon systems. CTS HiFi Cas9 was used with the TrueGuide β2M sgRNA at a molar ratio of 1:2. Cells were resuspended in T buffer for the Neon NxT system or CTS Xenon LCE Buffer for the Xenon system, and an electroporation program of 2,200 V with a single pulse of 20-millisecond pulse width was used across both platforms. Further details on the experimental parameters and conditions can be seen in Figure 14.

5 days post-electroporation, percent live cells and KO efficiency were measured via flow cytometry. As depicted in the plots in Figure 15, which compare control cells to cells containing the payload at the time of editing, the β2M KO efficiency was measured within the double-positive CD45RA and CD62L naive T_{SCM} population.

The CTS Xenon LCE Buffer demonstrated successful Cas9 RNP delivery and resulted in a scalable 15% β2M KO using higher energy electroporation parameters. The total viable edited cells amounted to approximately 4% of the electroporated samples (data not

FIGURE 9

Bar graphs depicting the transfection efficiency of GFP mRNA with appropriate controls that were either untreated or subjected to only electroporation or mRNA incubation.

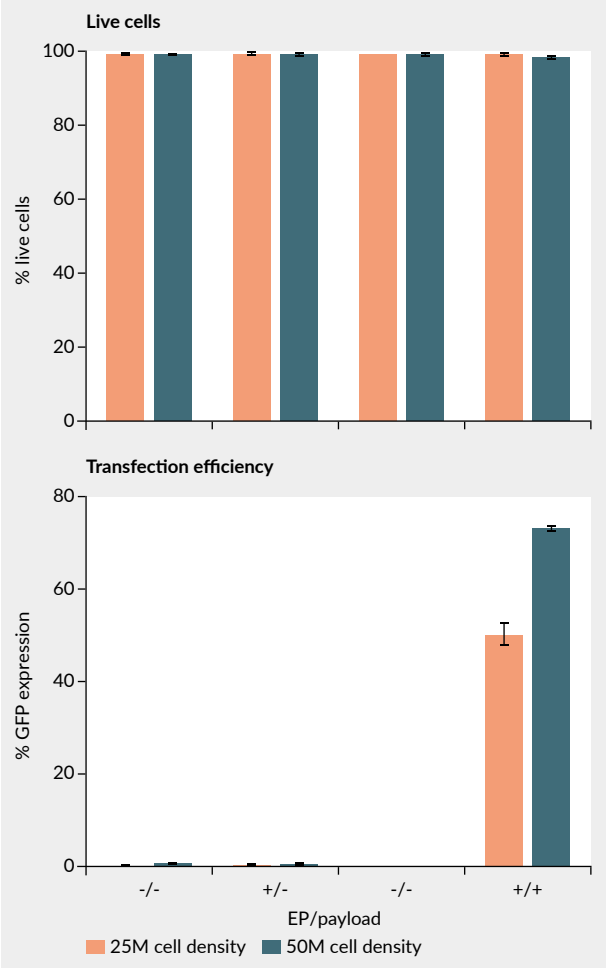


FIGURE 10

Phenotype maintenance post-electroporation: naivety and stemness markers (CD45RA/CD62L) post-GFP mRNA delivery.

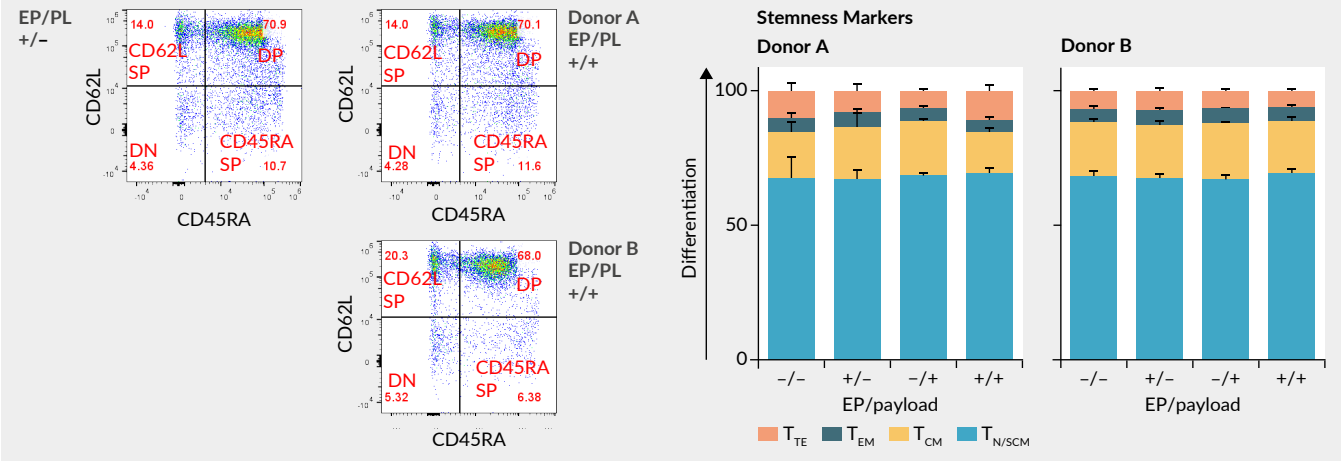


FIGURE 11

Activation markers (CD25/CD69) post-GFP mRNA delivery and phenotype maintenance post-electroporation.

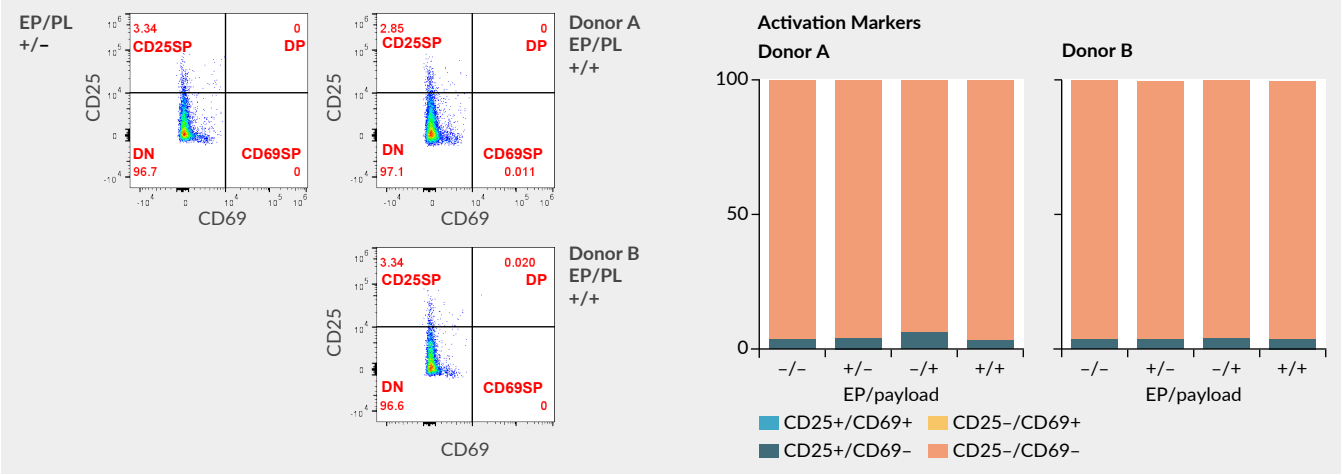


FIGURE 12

Experimental parameters and conditions of GFP plasmid delivery.

Experimental details	
Cell source and isolation	PBMCs were isolated from fresh leukapheresis products from two healthy donors using the CTS Rotea system; Pan T cells were isolated from PBMCs using Dynabeads Untouched Human T Cells Kit
Samples	N=2 donors
Concentration of cells during electroporation and electroporation scale	CTS Xenon Electroporation System: 25×10^6 or 50×10^6 cells in 1 mL SingleShot
Payload	eGFP 5 kb plasmid (5 µg/1 × 10^6 cells)
Buffers	CTS Xenon Lower Conductivity Electroporation Buffer
Electroporation protocols	CTS Xenon Electroporation System (2200 V, 20 ms, 1 pulse)
Culture vessel and post-electroporation seeding density	24-well plates; 1×10^6 cells/mL

shown), suggesting that enrichment or sorting techniques may be necessary for use in both functional testing and manufacturing downstream. Gene editing also maintained the stemness phenotype compared to the control samples, as evidenced by the flow plots and T cell subset populations shown in Figure 16.

The final datasets shown here cover transgene KI in resting T cells in two biological donors. Cells underwent electroporation using either the Neon NxT or Xenon electroporation systems to demonstrate scalability between two systems. The CTS HiFi Cas9 protein was employed alongside the TrueGuide TRAC sgRNA at a molar ratio of 1:2, with a 2.4 kb linear double-stranded (ds)DNA anti-CD19 CAR construct serving as the donor template. T buffer and CTS Xenon LCE Buffer were utilized on the Neon NxT and Xenon systems respectively, with the higher energy electroporation setting of 2,200 V and a single pulse of 20-milliseconds pulse width. The experimental parameters

FIGURE 13

GFP plasmid expression and transfection efficiency within the naïve CD45RA and CD62L expression populations in control cells versus cells electroporated with a payload across two donors.

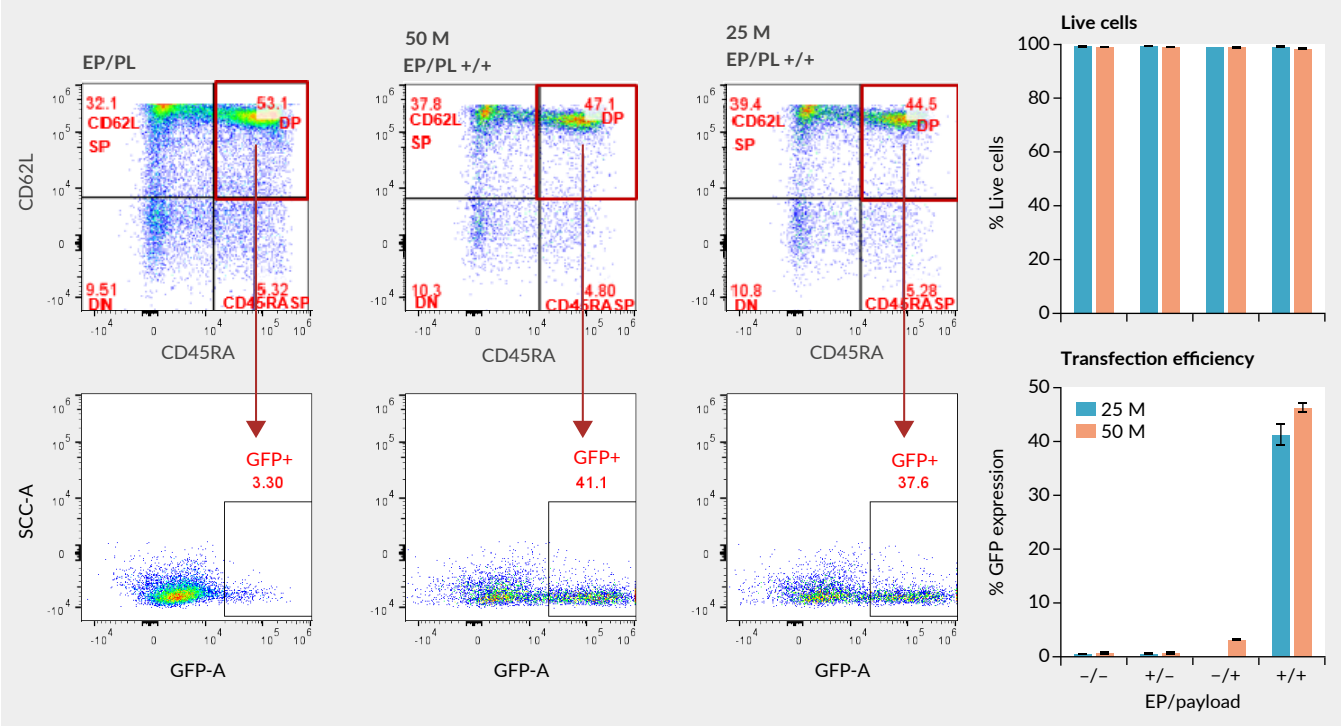


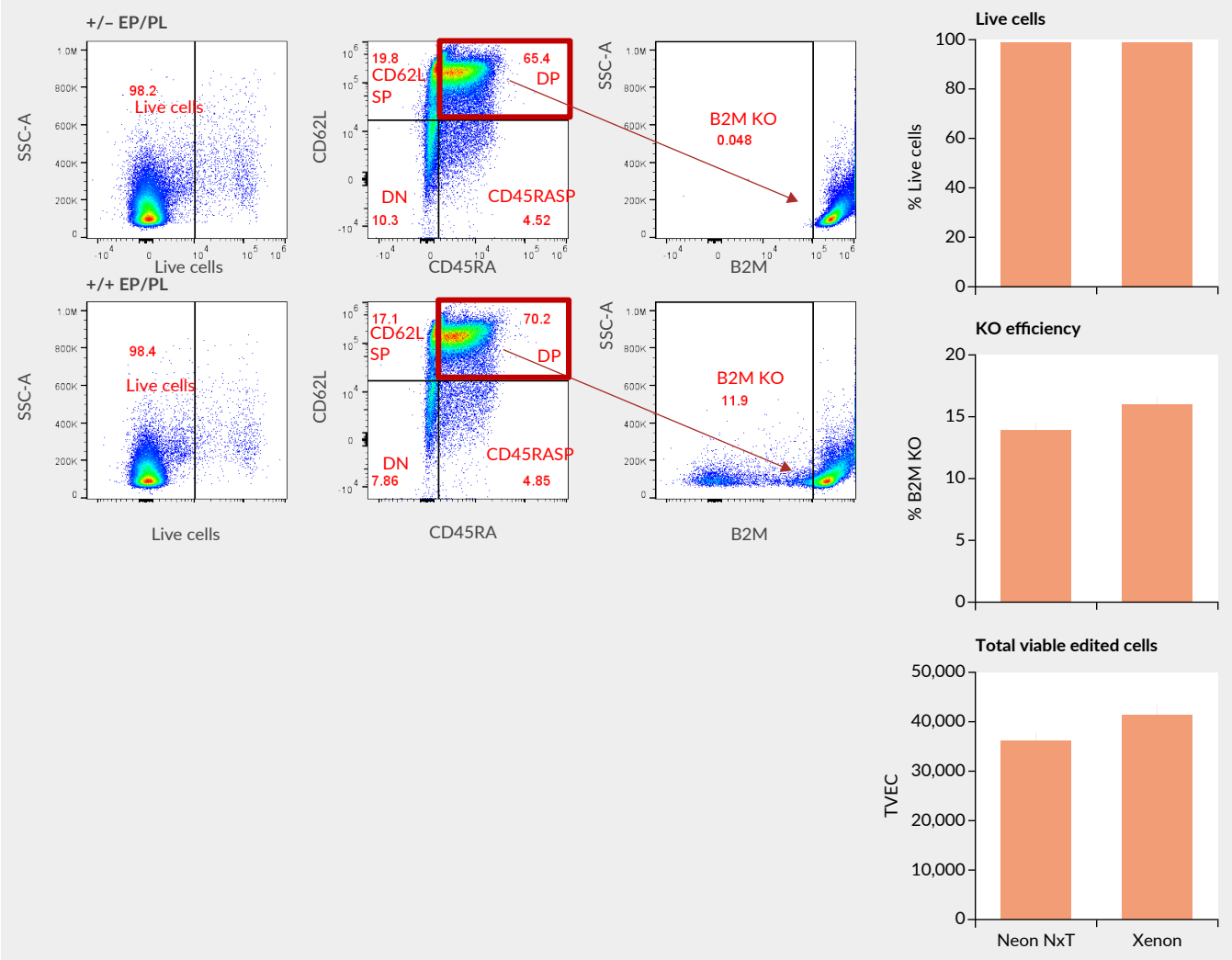
FIGURE 14

Experimental parameters and conditions for the KO of β2M for the non-viral gene-edited cell therapy manufacturing workflow.

Experimental details	
Cell source and isolation	PBMCs were isolated from fresh leukapheresis products from two healthy donors using the CTS Rotea system; Pan T cells were isolated from PBMCs using Dynabeads Untouched Human T Cells Kit
Samples	N=2 donors
Concentration of cells during electroporation and electroporation scale	Neon NxT Electroporation System: 5×10^6 cells in 100 µL tip CTS Xenon Electroporation System: 50×10^6 cells in 1 mL SingleShot
Payload	CTS HiFi Cas9 protein TrueGuide B2M sgRNA (Cas9:gRNA molar ratio 1:2)
Buffers	Resuspension Buffer T or CTS Xenon Lower Conductivity Electroporation Buffer
Electroporation protocols	Neon NxT Electroporation System and CTS Xenon Electroporation System (2200 V, 20 ms, 1 pulse)
Culture vessel and post-electroporation seeding density	24-well plates; 1×10^6 cells/mL (non-activated)

FIGURE 15

Percent live cells and knockout efficiency of β2M across double-positive CD45RA and CD62L post-electroporation.



and conditions for the KI in resting T cells can be seen in Figure 17.

11 days post-electroporation, percent live cells, KO efficiency, KI efficiency, and total viable edited cells were measured via flow cytometry. Figure 18 represents the control versus edited samples from both donors, examining TCR expression within the naive population and percent CD19 CAR expression based on V5 tag expression.

The CTS Xenon LCE Buffer facilitated gene editing while maintaining viability up to 11 days post-electroporation. It also enabled up to 20% TCR KO in the first biological donor, and up to 30% KO in the second donor. CD19 CAR integration efficiency of approximately 2%–6% was observed across both donors. The total viable edited cells amounted to approximately 4%–6% (data not shown) of the initially electroporated population, suggesting that, again, post-electroporation cell enrichment or cell sorting techniques may be necessary for both functional testing and manufacturing downstream.

The CTS Xenon LCE Buffer maintained the stemness of the electroporated sample compared to the controls across different T cell subset populations. Additionally, it enabled activation-agnostic gene editing

FIGURE 16

Stemness phenotype (CD45RA/CD62L) post-Cas9 RNP delivery for β2M knockout using higher energy electroporation parameters.

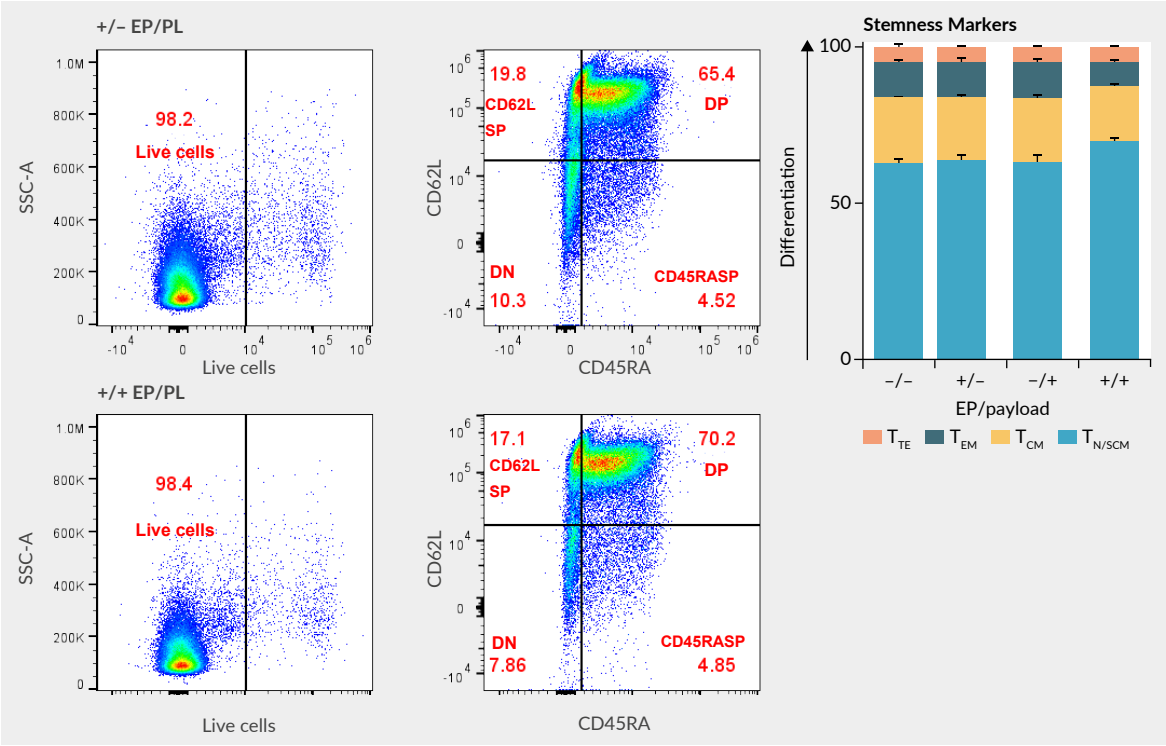


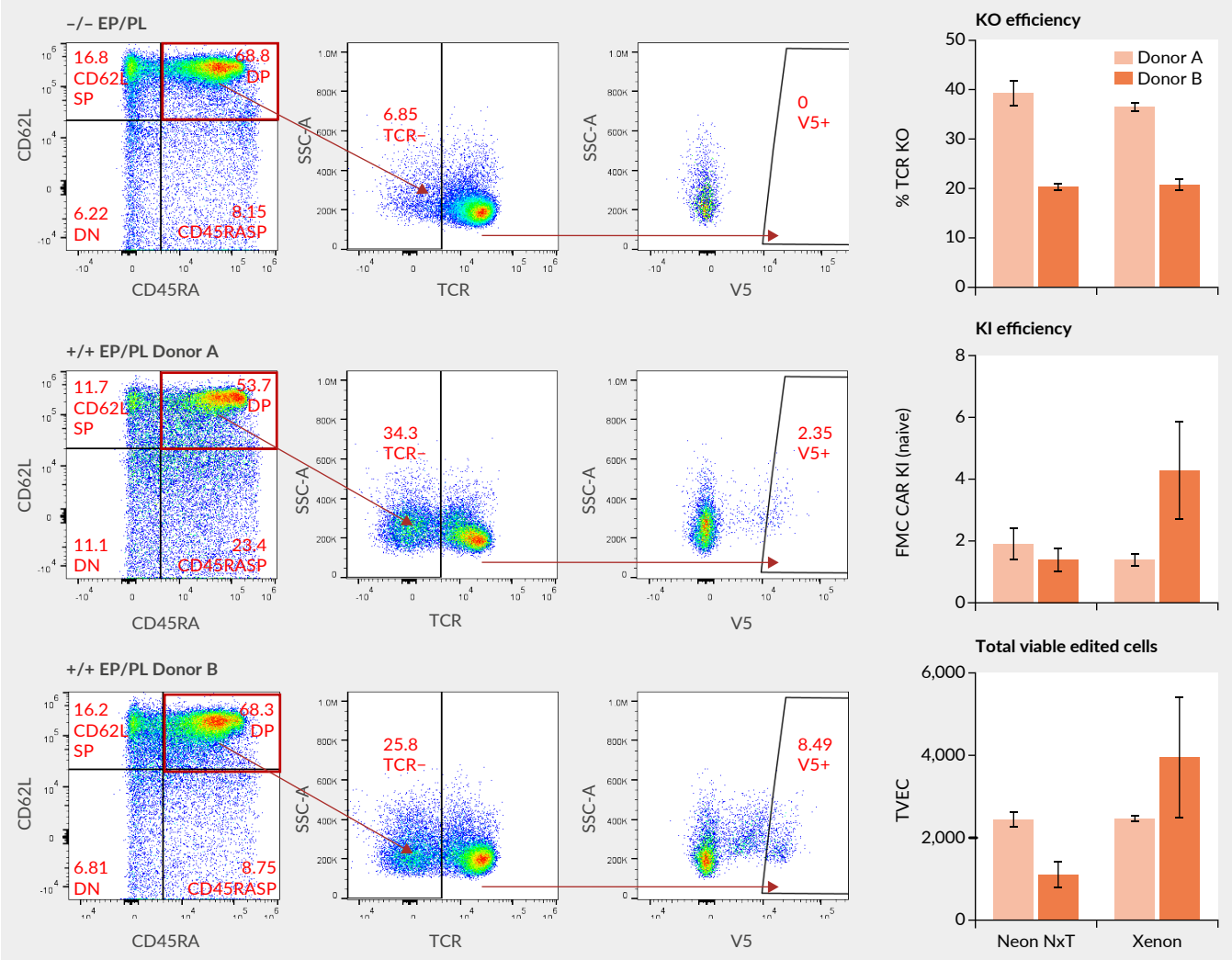
FIGURE 17

Experimental parameters and conditions for the knock-in of a linear dsDNA Anti CD19 CAR construct in a non-viral cell therapy manufacturing gene editing workflow.

Experimental details	
Cell source and isolation	PBMCs were isolated from fresh leukapheresis products from two healthy donors using the CTS Rotea system; Pan T cells were isolated from PBMCs using Dynabeads Untouched Human T Cells Kit
Samples	N=2 donors
Concentration of cells during electroporation and electroporation scale	Neon NxT Electroporation System: 5×10^6 cells in 100 µL tip CTS Xenon Electroporation System: 50×10^6 cells in 1 mL SingleShot
Payload	CTS HiFi Cas9 protein TrueGuide TRAC sgDNA (Cas9:gRNA molar ratio 1:2) 2.4 kb linear dsDNA Anti CD19 CAR–donor DNA
Buffers	Resuspension Buffer T or CTS Xenon Lower Conductivity Electroporation Buffer
Electroporation protocols	Neon NxT Electroporation System and CTS Xenon Electroporation System (2200 V, 20 ms, 1 pulse)
Culture vessel and post-electroporation seeding density	24-well plates; 1×10^6 cells/mL (non-activated)

FIGURE 18

Percent live cells, KO efficiency, KI efficiency, and total viable edited cells post-dsDNA CAR construct KI in resting T cells.



of resting or non-activated T cells without inducing specific early or late activation markers, as evidenced in the representative flow plots and graph in Figure 19.

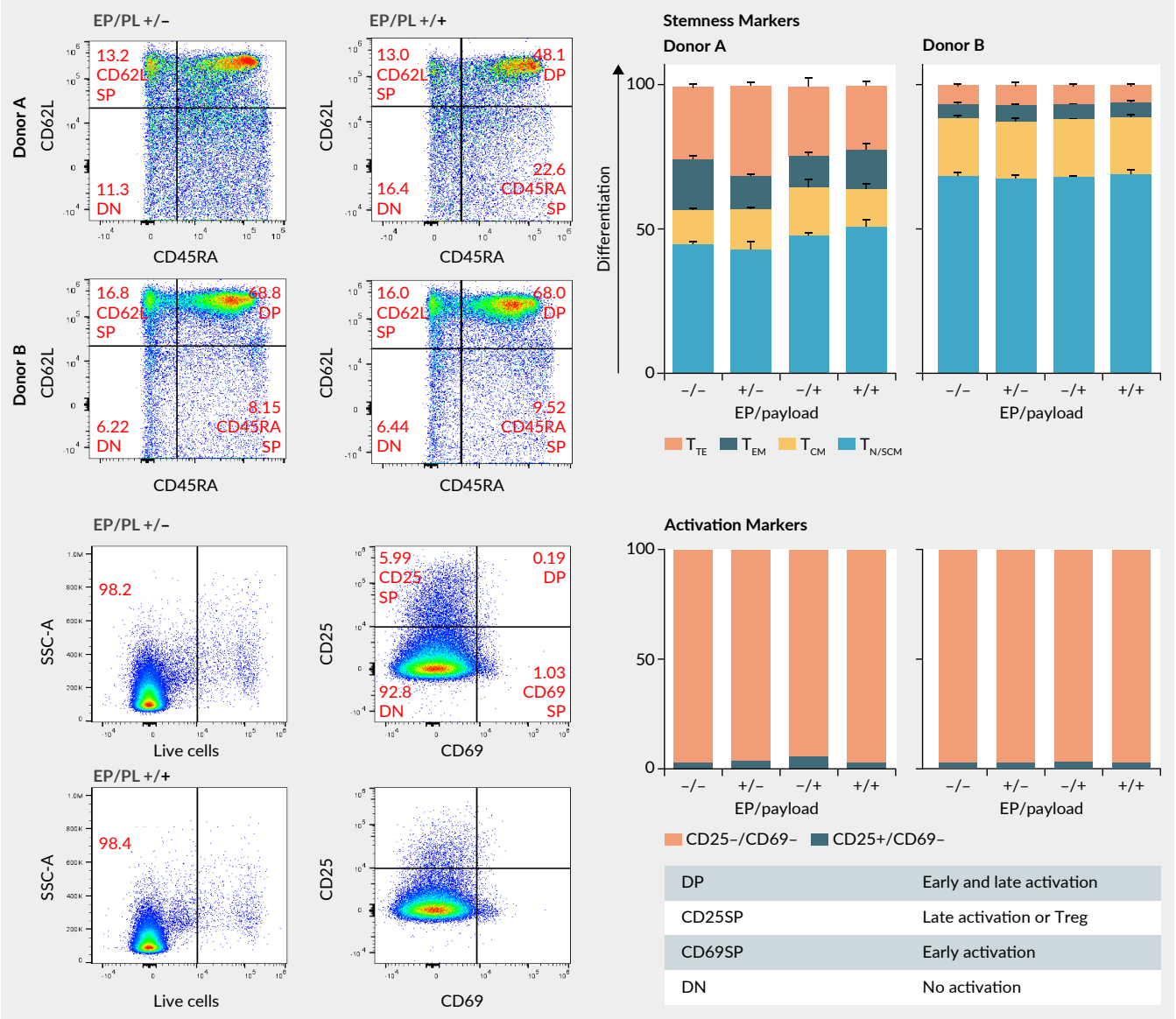
SUMMARY

To conclude, these studies demonstrated that the expanded capabilities of the CTS Xenon electroporation system in combination with the CTS Xenon LCE Buffer for transfection of resting, non-activated T cells. While additional optimization can improve EP and KI efficiencies, a workflow was established for engineering these hard-to-transfect cell types utilizing the CTS Xenon LCE Buffer. This advancement enabled activation-agnostic transgene insertion in resting T cells.

Overall, the integration of the CTS Xenon LCE buffer in the Xenon toolbox enables a wider design space for CAR-T developers and can allow cell therapy manufacturing customers to expand Xenon system utility to other cell types and applications.

FIGURE 19

Stemness (CD45RA/CD62L) and activation markers (CD25/CD69) post-transgene knock-in.



DP	Early and late activation
CD25SP	Late activation or Treg
CD69SP	Early activation
DN	No activation

Q&A

Akshaya Chandrasekaran

Q Have positive selection kits been used for T cell isolation?

AC: No, we have tried using other negative selection kits that enrich for a more naive-like population, and the resulting KI efficiency is similar to that observed using a negative selection kit. With this workflow, the non-activated, non-proliferative nature of the starting T cell population makes them harder to transfect. However, the CTS Xenon LCE Buffer facilitates this specific transfection. That said, we will have upcoming data sets using more positive selection kits, which we can seamlessly integrate into a manufacturing workflow.

Q Do naive cells expand after electroporation?

AC: The naïve cells do not expand unless they are exogenously activated and remain in a non-activated, non-proliferative state. It is necessary to enrich the specific gene-edited population and activate them exogenously to achieve an expanded population suitable for downstream applications.

Q What is the total process recovery (total input cells versus total viable edited cells)?

AC: As long as the cells remain non-activated, we achieve a 4%–8% recovery based on the input population versus the final edited cell counts that are viable. This recovery rate can be improved by activating these cells post-electroporation. Activation will facilitate the expansion necessary to obtain enough cells for scale-up and manufacturing, and we hypothesize

improved integration of the transgene through induction of the homology-directed repair pathway.

Q How can total process recovery be improved?

AC: There are two strategies we could employ. Firstly, we could activate the cells using the CD3/CD28 beads post-electroporation. This approach is anticipated to enhance the overall quality of the CAR-T cell product by minimizing chromosomal losses and translocations, thus presenting a safer option for clinical use.

The second option involves inducing a challenge *in vivo*, utilizing mouse or other animal models. In this scenario, naive T cells would be delivered and subjected to continuous antigenic stimulation *in vivo*, leading to proliferation and activation [1]. This method is how we would test for functionality.

Q Have you experimented with multi-pulse programs with the low conductivity buffer?

AC: Yes, we have explored programs with slightly shorter pulse widths and increased pulse numbers and compared them to longer pulse width in a single pulse. With the CTS Xenon LCE Buffer and the expanded range of programs available on the Xenon Electroporation System, performance remains comparable, providing we adhere to the energy limits supported by the CTS Xenon LCE Buffer.

For instance, employing a 2,200 V, 20-millisecond single pulse versus four pulses of 2,200 V with a 5-millisecond pulse width would yield a similar result. The choice between approaches is primarily dictated by the specific application. This means that early identification via process development of whether a longer single pulse width with improved efficiency or distributing the applied energy across multiple pulses resulting in higher total viable edited cells at the end of the process is crucial.

Q Can this process reduce secondary malignancies? Further to that, could you comment on the low conductivity buffer and the significance of that?

AC: Secondary malignancies are particularly important as the field is increasingly focusing on using naive T cells. It is notable that this population, while resting and non-activated, retains its genomic integrity and capacity for proliferation and differentiation *in vivo*, therefore presenting a potential strategy for addressing tumor relapse.

Regarding the CTS Xenon LCE Buffer, it is crucial to consider that non-activated T cells are notably smaller than their activated counterparts. It is understood that their membranes are more compact, necessitating a higher energy setting for effective membrane penetration and transfection. By utilizing a lower conductivity buffer, the overall energy within the system is modulated, enabling the use of higher energy programs without compromising cell health and

recovery. This is why employing the CTS Xenon LCE Buffer enables effective transfection of resting and quiescent cell types.

Q Can you speak to current Xenon buffer offerings and format?

AC: There are currently three buffers available for use on the Xenon electroporation system. These include the Gibco™ CTS™ Xenon™ Electroporation Buffer, the Gibco™ CTS™ Xenon™ Genome Editing Buffer tailored for specific KI applications, and the CTS Xenon LCE Buffer. All three are parts of the CTS solutions portfolio and are offered in two formats: a 100 mL bottle format most often used in process development, and a 100 mL bioprocess container (a bag format) designed for seamless scale-up and cell therapy manufacturing applications.

Q What electroporation parameters, such as voltage, pulse length, or waveform shape, are used for non-activated T cells?

AC: For the CTS Xenon LCE Buffer, higher energy settings are employed. This involves a combination of voltage, pulse width, and pulse number resulting in significantly higher energy levels compared to those achieved with other Xenon buffer options.

The Xenon Electroporation System results presented here used a single pulse of 2,200 V with a 20-millisecond pulse width. This extended pulse width is facilitated by the CTS Xenon LCE Buffer. This in turn allows for longer pulse widths without the risk of arcing or adverse effects on cell health. While in these experiments we utilized a single pulse, we have also explored multi-pulse programs to distribute the high energy across several pulses rather than a single pulse.

Q Do you have benchmarking data of GFP expression using reference controls, particularly for monitoring efficiency over several days post-electroporation?

AC: Although we have not yet done a direct comparison, we have compared efficiency using different buffers. In order to ascertain that the LC buffer is optimal for non-activated T cells, we have compared it with other buffer options. It is worth noting that employing a higher energy setting with some buffers is not permissible due to system limitations.

We have observed that using the CTS Xenon Electroporation Buffer or the CTS Xenon Genome Editing Buffer at this higher energy setting may result in arcing, however, employing the CTS Xenon LCE Buffer expands the array of programs or parameter combinations that can be tested. This enhances control over process optimization and development, particularly for cell types that are more challenging to transfect.

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BIOGRAPHY

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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT

INTERVIEW

How will recent agency initiatives and likely future directions for regulatory CMC guidance impact the cellular immunotherapy space?

It has been a busy past year or two in terms of regulatory evolution and initiatives that impact cellular immunotherapy developers. **David McCall**, Senior Editor, BioInsights, invites **Adeyemi Afuwape** to unpack some key pieces of recent guidance on the regulatory CMC side, and to share his thoughts on what the future may hold in this regard.

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Q What are you working on right now?

AA: I was until very recently working at Resolution Therapeutics (RTx) on their first regulatory submission for a Phase 1/2 study of their first-in-class, gene-modified cell therapy.

This is an engineered macrophage-based advanced therapeutic targeting end-stage liver disease. I lead the compilation of the CMC package and the overall Clinical Trial Authorisation (CTA) submission via Combined Review. Prior to this, RTx engaged in Scientific Advice meetings with a number of EU national Competent Authorities to support this clinical trial. Subject to the CTA being approved, RTx is aiming to commence the clinical trial by the end of this year, which is really exciting.

Q What for you have been the key recent developments in regulatory guidance for the cellular immunotherapy space, and why? How will they impact cell and gene therapy developers and manufacturers?

AA: There have been several that have caught my eye lately, predominantly from the US FDA.

The first thing that jumped out to me was the black box warning slapped on approved CAR-T cell therapies in January 2024. This happened because there were some signals of secondary T cell malignancies in the long-term follow up (LTFU) of these CAR-Ts. This applies to all of the approved BCMA and CD19 CAR-Ts with the sole exception of Tecartus®, where no such signals were observed. I don't think there is any question that the risk–benefit analysis is still very good for the commercial CAR-T cell therapies—we are talking about 22 cases out of more than 30,000 patients who have now been dosed. What the FDA has done, though, is to take a hard look at the LTFU for these therapies and as a result of this review, have extended the previous standard of 15 years of follow-up to lifelong assessment. I think this is going to be really important for the field in terms of the CMC package because it actually updates the specifications and the information required. In a report in *Nature Medicine*, Drs Bruce Levine and Carl June (University of Pennsylvania) commented on this development and they concluded that the FDA was perhaps a bit overzealous in extending LTFU to lifelong assessment as the risk of secondary malignancies following CAR-T treatment is arguably extremely small. Still, I think it brings the issue of LTFU very much to the fore in the CAR-T space.

FDA also released a guidance, Safety Testing of Human Allogeneic Cells Expanded for Use in Cell-Based Medical Products, in April 2024. That is important because it really acknowledges the advent of allogeneic cell therapy products that are coming through. More specifically, the FDA is looking to emphasize the need for acceptable limits for identity, quality, and purity of the investigational medicinal product. There are clearly going to be some justifiable limits put in place to control these aspects. They are also looking at the key aspect of genomic integrity of these products, and generally making sure that there is a good characterization background for the future growth of the field.

The FDA also looked at the two-tier system for testing both Master Cell Banks (MCB) and Working Cell Banks (WCB), particularly focusing on the aspects that only apply to allogeneic cell products as opposed to biologics development in general. One thing that stuck out for me was that they have expanded the testing of donor starting material from what was laid

"...the onus [is] now firmly on demonstrating that the number of edits is justifiable and appropriate for a given patient population."

out in 21 CFR 1271 Subpart C. For example, they have included testing for cytomegalovirus (CMV), Epstein-Barr virus (EBV), parvovirus B19, and the human herpes viruses, HHV-6, 7, and 8, none of which were specified as being mandatory before. It is interesting that they also included John Cunningham (JC) virus. So, they are clearly now looking at expanding the range and number of communicable agents that need to be tested, which I think shows a maturation of the regulatory guidance and the allogeneic cell therapy field in general. They also focused on genome editing for retroviral testing. Here, they are exploring the amount of read depth required to look at off-target and on-target edits and integration sites, as well as the usual assessments of cytogenic testing, karyotyping using G-banding, and tumorigenicity.

Another important guidance that FDA released in the beginning of the year was Human Gene Therapy Products Incorporating Human Genome Editing (January 2024), which looked at the way to control the gene editing components: the guide (g)RNA, the DNA templates, and the ribonucloproteins complexes. FDA was also concerned with aspects relating to the specific delivery system involved, for instance, whether that be plasmid DNA, lentivirus, or AAV. The Agency also wanted to look at the classification of the degree of modification for the cells for the given patient population, in order to ensure that whatever gene editing is being carried out fits optimally with the specific patient population or indication in question. The frequency of editing, or the therapeutic editing threshold, was a further area of interest for FDA here. Essentially it is an analysis of the number of edits being carried out versus the number of cells involved, with the onus now firmly on demonstrating that the number of edits is justifiable and appropriate for a given patient population. Developers are also required to monitor the indels—the DNA insertions and deletions—which should be calculated from clinical data.

In the context of *ex vivo* gene editing of cell therapies, the FDA also examined the components of the genome editing tools as critical starting materials, and the corresponding need to assess their quality. There is a real emphasis now on looking at the manufacturing process of these components, what kind of testing is expected for them, and whether that testing is sufficiently fit for qualification purposes.

Finally, in December 2023, the FDA released the guidance Potency Assurance for Cellular and Gene therapy Products, which discussed the strategy for quality risk management when developing a potency assay.

The FDA normally considers the issue of when to introduce a potency assay as a phase-appropriate decision, especially for cell and gene therapy products. However, this is with the understanding that it would need to be in place by the time a developer is planning the registrational/pivotal study at the latter stages of development. In Europe, it is slightly different, as there is an expectation that cell and gene therapy developers have a potency assay from the outset of clinical development. In contrast to the USA, it wasn't necessarily a requirement to have a potency assay in place for a Phase 1/2 trial. However, I feel there has been a subtle

change because the FDA is now emphasizing to developers that they need to start their potency assay development as early as possible. They are asking developers to look at the risk assessment concerning potency, how it links to the mechanism of action, and to then demonstrate that in development. It basically demands a sound control strategy: knowing the critical quality attributes (CQAs) and critical process parameters (CPPs) of the product, risk assessing what the product is, then having a risk assessment in place for how the developer would show that their potency assay is fit for purpose and will be phase-appropriate throughout development.

One interesting thing about this (which was actually reflected in our recent approach at Resolution Therapeutics) is that the FDA is saying it is not unusual for developers to begin with a matrix of different potency assays. Then, as they risk assess and continue development, they would hone it down and follow a process of elimination until they arrive at an assay(s) that is most suitable and relevant for the given indication.

Q Are there any specific areas of divergence in national or regional regulations affecting the space that are of particular concern to you, and why?

AA: Yes, and I think, by and large, that it is the same old differences between the USA and the European Union (EU). For example, in the USA, drug master files (DMFs) are available, which are especially useful early on in development to control the components of product manufacture. However, in the EU, these are not necessarily recognized. As a developer, it is imperative for you to show that you know the quality of your raw and starting materials and have an understanding of what the manufacturing process is for them, and the testing involved—for example, concerning viral or adventitious agent testing. Without a DMF, that sort of information can be very hard to come by, because the suppliers of these materials have to protect their IP and their commercial advantage and interests. There is a disconnect there.

If you are starting a clinical trial in the USA, you can use a Letter of Authorization to get access to a particular DMF, and the IP is protected. In Europe, however, you have to talk to the suppliers about a regulatory strategy to ascertain precisely what information they will and won't allow you to use, and what is going to be most important to allow you to move forward. Ultimately, it is incumbent on the developers to prove the quality and the safety of their product, which extends to the raw materials being used, so I think this is a really key area of difference for those developing advanced therapies in Europe to consider. It is important to note that by the time of licensure, both FDA and EMA will expect the developer to have a full account of the quality and safety of all starting, raw, and ancillary materials in the BLA and MAA, respectively.

The other key difference for me would be the GMO regulations in Europe, which don't exist in the USA—this has been a longstanding issue, but unfortunately, it is one that isn't going away. There are two things of note here—firstly, the GMO application runs alongside the CTA submission, but depending on which EU national competent authority is involved,

you could be dealing with one agency for both (as is the case in France with the Agence Nationale de Sécurité du Médicament et des Produits de Santé (ANSM), and Germany with the Paul-Ehrlich-Institut), or you could be dealing with two separate entities, as is the case in the UK, Spain, and The Netherlands. Working with a single agency generally means shorter and more importantly, aligned timelines, so there is a heterogeneity in terms of receiving decisions/approvals across different European nations, which obviously can have an unwelcome impact on your clinical trial plans.

Secondly, there is the question of whether your product is going to be categorized as being under 'deliberate release' or 'contained use', which have different requirements for information. 'Deliberate release' will involve a lot more scientifically detailed information whereas for 'contained use', the information needed is more administrative, involving consultation with each individual clinical site within the trial.

There has been some harmonization with the GMO regulation paperwork in that there is now a Common Application Form (CAF) required for all GMO applications for participating EU jurisdictions. For 'deliberate release', the Summary Notification Information Format (SNIF) is necessary to show the environmental impact to adjoining countries. But it does remain rather cumbersome, still adds a lot of time, and makes it difficult to predict CTA submission and overall clinical trial timelines. Neither this extra time nor the resulting uncertainty are good for the developer from a fundraising perspective. Therefore, it is important to ensure that strong project management is in place to handle these submissions.

Q The upsurge in applications of cellular immunotherapy in the autoimmune disease area is a major current trend with some companies pivoting away from oncology. What are the key related considerations here from a regulatory CMC perspective?

AA: Quite a lot of companies have pivoted in this way lately, but I do think that there are a lot of things that can be taken from the oncology space. The most obvious thing is that you are using the same basic methodology—ablating or depleting B cells. To some extent, it is possible to show either remission in certain cases or comparable efficacy signals, and a favorable safety profile that is comparable to oncology.

I think two things will be really important from a CMC perspective. Firstly, with the B cells that are ablated to stop the production and release of autoantibodies, it will be key to look at whether the dose would be comparable to that for an oncology indication. From what I have seen, the dosages being used in autoimmune disease indications tend to be slightly lower than those used in oncology. For example, the approved CD19 CAR-Ts in oncology dose range from $1–5 \times 10^6$ cells/kg, whereas for the autoimmune conditions, they are typically using a dose of 1×10^6 cells/kg—so, there is some difference there.

Secondly, I think there is also a need to look at the reoccurrence of B cells in patients. In the oncology setting, the patients will need long-term immunoglobulin treatment due to the

"...the safety assessment process for autoimmune disease-targeted CAR-Ts is likely to attract more regulatory scrutiny."

permanence of the B cell removal. But early observations from recent trials show that this is not necessarily true for autoimmune conditions where over time following B cell ablation, normal non-pathological B cells are reconstituted and replenished, without autoreactive B cells being present. This raises important questions around the safety profile and persistence of these CAR-T cells—for instance, would they extend the knockout or removal to non-autoreactive or normal B cells over time, and is this dose dependent?

Obviously, with the fact that the level of morbidity for oncology indications is going to generally be so much higher than it is for autoimmune diseases, the safety assessment process for autoimmune disease-targeted CAR-Ts is likely to attract more regulatory scrutiny—also because of the relative number of alternative treatments available. This will impact issues of safety profile and assessment and/or LTFU requirements, which will most likely be more stringent than those required for oncology.

Additionally, regarding the potency assay, some autoimmune conditions such as lupus erythematosus can be highly systemic. This raises the issue of how to regulate potency to show that it is actually linked to a mechanism of action. Removing the autoreactive B cells is one thing; what does that mean for the actual underlying disease, and therefore, how does that relate to the potency assessment of the final drug product? Consequently, I think that linking potency to mechanism of action is going to be a little more complex in systemic autoimmune indications than it would be for oncology.

Finally, I think any assumption that the CD19 CMC packages for autoimmune conditions would be equivalent to that required for hematological indications would be misplaced. In some regards, it is a starting template, but there are a lot of things that have to be looked at specifically such as distribution, safety profile, even dose. Usually, there would be some benefit of hindsight from similar CD19 CAR-Ts having been approved in oncology indications, but I think it will be a case of starting from scratch with a different idea when it comes to the treatment of autoimmune indications with CD19 CAR-T.

Q How are industry regulatory CMC compliance strategies evolving to address the ever-increasing complexity of engineered cell therapy products—and are there any particularly pressing gaps in our knowledge or the available analytical toolkit, for you?

AA: The thing that jumps out for me here is iPSC-derived cells, which a lot of developers are now turning to. There are several aspects to this, including the need to develop separate testing that demonstrates safety at the MCB and the allogeneic cell therapy product levels.

Tumorigenicity is one of the specific things that I think is an issue because of the need to balance looking for the safety of the product with expediency of the overall development. Time is money. But while it is important to find a way to test for tumorigenicity in a time-efficient and economical manner, the gold standard way of doing this—*in vivo* animal studies—currently involves at least a 6-month wait for readout in immunodeficient mouse models.

The advent of analytical tools like droplet digital (dd)PCR helps here, as it can not only provide an adequate means of measuring the tumorigenicity of an iPSC-derived product as well as undifferentiated cells (a really important impurity in the context of the safety profile), but it also helps with both the timeline and with sensitivity. The latter is particularly important when you are working with what I would term 'valuable material': it is obviously preferable to use as little of that material as possible for testing purposes. As discussed earlier with the guidance on Safety Testing of Human Allogeneic Cells Expanded for Use in Cell-Based Medical Products, in terms of the establishment of MCB/WCB, the conundrum of where to incorporate GMP manufacture can prove to be challenging. Can nonclinical/preclinical data and risk assessment provide justification for performing pluripotency reprogramming alone or with genome editing under GMP-like conditions (the obvious advantage being that it is financially cost-effective to introduce these early steps at a research facility or a site that at least adheres to GMP principles) only to later introduce manufacturing under full GMP, possibly at a CDMO, to establish the MCB with the required genome edits? This can be fundamental in producing multiple drug products from a single MCB as a form of platform technology. These are definitely CMC questions that I recommend be discussed very early in meetings with regulatory agencies (INTERACT [FDA], ITF [EMA], IO [MHRA]) before embarking on the development of a manufacturing process that could be difficult to reverse or revise if deemed not to be appropriate or adequate.

Q How and where specifically can off-the-shelf therapy developers leverage the fund of autologous cell therapy-derived CMC knowledge to help advance the allogeneic cell therapy field?

AA: I think the first thing to bear in mind is that the autologous cell products have a lot of dispensation in the sense that there are a lot of justifications provided simply because of the imperative to manufacture and QC/QA the treatment and get it back to the patient as rapidly as possible. So, there is a certain level of leeway given—for instance, in the implementation of routine rapid sterility testing as part of drug product release. The allogeneic cell therapies will not benefit in the same way because they are off-the-shelf in nature and not as time restricted and/or sensitive.

However, what we have seen recently is some of the approved BCMA CAR-Ts having their approvals modified from third-line or fourth-line to second-line treatments. That means that, in general, the T cells collected from patients will be of better quality and less exhausted or sick than was previously the case. Allogeneic cell therapy developers will be able to look at efficacy

and safety aspects in terms of how those autologous therapies are performing and draw more meaningful parallels with their own products, because despite being still different, the healthy donor cells will have more in common with these healthier patient cells. The question for regulators will then be: 'What to do now in terms of looking at the mechanism of action, potency assessment, and the feedback from clinical data?' What I think we will see there is that if there is any pushback from the regulators in terms of the CMC hurdles the autologous cell therapies need to clear as they progress into second-line or even first-line treatments, that will definitely impact the allogeneic cell therapy products too, as they will be expected to at least meet the same regulatory bar. It will therefore be crucial for allogeneic cell therapy developers to closely monitor the number of autologous CAR-Ts that continue to move up the treatment line in the knowledge that they are likely to have to clear a slightly higher CMC barrier with their own allogeneic products.

Regarding the non-T cell off-the-shelf products such as NK cell therapies, there is less they can leverage on the safety profile side of things because NK cells don't involve cytokine release syndrome or neurotoxicity, for example. I think what developers of those products can do is to look at how the autologous T cell products demonstrated their benefit.

The other key aspect to consider for allogeneic cell therapies is that they potentially could be involved in redosing. The FDA has provided another guidance Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products in January 2024, which we didn't discuss previously, regarding instances where patients may have previously received a CAR-T therapy. In those cases, the emphasis is going to be on measuring and monitoring any lingering presence of the previous CAR-T cell therapy in the patient's blood or tumor samples and understanding what effects and/or influences that they may have on the second CAR T therapy. This may well be a relatively common occurrence in the future with allogeneic cell therapies potentially being indicated to follow an autologous treatment, i.e., in cases where a patient is unable to undergo further leukapheresis for a second treatment.

Q Looking to the future, what do you expect to see next in the way of regulatory guidance evolution for the cellular immunotherapy field?

AA: One thing that stands out for me is yet another recent initiative by the FDA, which they introduced in December 2023—the Advanced Manufacturing Technologies Designation Program. Advanced therapy developers are always focused on reducing their cost of goods sold (COGs), which obviously involves optimizing their processes and increasing automation. Consequently, there is a steady stream of novel, integrated manufacturing technologies being introduced that seek to reduce manual unit operations, cost, and timeline of both the manufacturing process and the overall vein-to-vein supply chain. This new designation program from the FDA seeks to assist developers in assessing how these novel and emerging technologies can help to improve their cell therapy product's quality and expedite its delivery to patients. I think

this shows that the FDA is getting ready for a massive increase in the number of developers looking to improve their manufacturing processes, reduce timeframes, reduce costs, and generally make their products more affordable for reimbursement.

New technologies are always going to be evident as the cell and gene therapy field matures. Looking ahead, I can imagine that regulators on both sides of the Atlantic will continue to look at supporting the integration of new manufacturing and supply chain technology, even following commercialization.

Q What does the foreseeable future hold for you?

AA: Currently I'm working as a freelance contractor advising cell and gene developers on their CMC regulatory strategy. However, I look forward to joining another advanced therapy company with a wide and exciting pipeline that has the same scientific innovation and ethos as Resolution Therapeutics. So, I aim to remain involved in the cutting-edge of advanced therapy CMC. It is such a crucial strategic aspect of drug development, affecting business development, clinical, etc. Also, I would like to help the field through interactions with the regulators: as more and more new technologies come in, so the regulators have to adjust their thinking and guidance, and I would like to be part of the testing and pushing of the boundaries of knowledge around that. I think that's what regulatory affairs, and especially CMC, is all about.

BIOGRAPHY

ADEYEMI AFUWAPE was Senior Director, CMC Regulatory Affairs at Resolution Therapeutics and was responsible for the CMC package of their first in class engineered macrophage cell therapy for the treatment of end stage liver disease (ESLD). This contributed to the submission of a CTA to initiate a Phase 1/2 clinical trial later in 2024. Prior to joining Resolution, he served as Group Head, Regulatory Sciences—ATMP at ProPharma Group where he led a team advising clients on early- to late-stage development of their advanced therapy products. Previously he held CMC regulatory consulting positions at ERA Consulting (now BioPharma Excellence, a PharmaLex company), Diamond Pharma Services, and PharmaLex. He gained his ATMP experience as Lead Process Development Scientist—Immunotherapy at the Cell and Gene Therapy Catapult in London. He has over 20 years of industrial experience as an immunological expert in medical devices and biologics with a strong focus on cell and gene therapies. Yemi holds both a BSc in Pharmacology from Kings College London, London, UK an MPhil in Pharmacology from the University of Sheffield, Sheffield, UK and a PhD in Immunology from Institute of Child Health, University College London, London, UK.

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CELL & GENE THERAPY INSIGHTS

INNOVATION IN CELLULAR IMMUNOTHERAPY: HOW TO REACH MORE PATIENTS?

SPOTLIGHT

A pathway forward for CAR-NK cell therapy

Scott McComb
National Research Council of Canada and University of Ottawa

"CAR-T works well in the hematological malignancies space, but it is still a short-term solution. I believe CAR-NK can be the long-term answer."

On Thursday, April 18, 2024 **David McCall**, Senior Editor, BioInsights, asked **Dr Scott McComb**, Research Officer, National Research Council of Canada and Adjunct Professor, University of Ottawa, for his views on current challenges and future prospects for NK cell therapy. This Viewpoint article is based on that conversation.

VIEWPOINT

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INTRODUCTION

I am a cellular immunologist by background. During my PhD at the University of Ottawa, I began studying apoptosis in T cells and the mechanisms by which T cell populations contract; why do 90% of T cells disappear after an infection? What controls that process? Then, for my postdoc, I went to a cancer biology lab to explore diseases of the immune system, particularly B-cell leukemia. Again, my focus was on cell death pathways in B-cells. That was the beginning of my strong interest in understanding what controls the dynamics of cell survival. More recently, my work at the National Research Council Canada has focused on CAR-T cell therapy development and specifically, the processes that allow CAR-T cells to proliferate, and to then respond to and outcompete a cancer. For me, the fundamental beauty and power of CAR-T cells is not simply that they can kill cancer cells—it is their ability to outgrow the cancer that makes them really special.

More recently still, my work has taken me beyond T cells and into other immune cell types, such as NK cells. Part of our mandate at the National Research Council Canada is to develop new therapies that can be affordable and accessible to Canadians in particular. But clearly, this is a global problem. If we want to see these therapies have a real impact in other cancer types and other regions of the world, we need to move beyond the currently unsustainable autologous CAR-T cell manufacturing model. NK cells offer us an opportunity to rethink the paradigm.

OBSTACLES TO PROGRESS IN NK CELL THERAPY R&D

As an outsider who recently arrived in the CAR-NK cell therapy field, it is important for me to acknowledge the considerable work that has gone before me. This is not a particularly new field of research. However, I believe there are a number of issues that have prevented the field from realizing its full potential as of yet.

My first observation is that a lot of NK cell therapy development is happening in the dark, which I think is really hurting the field. Too much research is going unpublished. There have been many clinical trials conducted over the past five years, however, relatively little data has been shared through the peer-reviewed literature to help us understand what went wrong in some of these trials, or to explain the preclinical development in detail. What has been interpreted by some as negative data is actually just a vacuum of data. For instance, how were the lead therapies identified in each case? How were these products developed? How did we assemble the different components?

NK cell therapies are somewhat more complicated than CAR-T cell therapies in that there is no standard approach. With T cells, you are largely confined to a small handful of proven techniques—for instance, to purify the cells using magnetic CD4 or CD8 beads. For T cell activation, you are almost always going to be using CD3/CD28 beads. While there may be a few different flavors of technology—multimers, for example—and a limited range of tools for transduction or transfection, it basically all comes down to a limited number of strategies. Thanks to the many years of development and numerous publications in CAR-T cell therapies coming out of academia, everybody now speaks a common language. However, in the NK cell therapy world, while there are certainly some great academic laboratories involved, it is not yet enough. We need more academic investment in NK cell research, and we need to see some of the things that didn't work being published. In particular, I think it is a travesty when clinical trials are conducted but the data is never released. Hopefully, some of this data will still be forthcoming in future publications, because I see this as one of the biggest problems facing the field. We need to learn more from each other, and we need to make sure that we are building a core population of researchers who are interested in building this space. We need the focus of both funders

and research institutions who really want to see the science of NK cell therapy develop.

What are the scientific problems they need to address? First and foremost, it is about the cells themselves. We need to understand how we can have more standard processes for manipulating NK cells. For example, there are many different flavors of feeder cells utilized in NK cell expansion and in some cases, non-cellular technologies such as beads are used. All of these different competing methods for manipulating the NK cells need to be tested and compared. The same goes for the transgene molecule being used: some of the most successful CARs have included IL-15, but there is not a great deal of data showing the same CAR with and without IL-15. So, different components are being assembled, but how they work individually is not necessarily being demonstrated. Again, there is a dearth of papers publishing this data to allow best practices to be established, and then built upon. The consequence of this is a familiar problem in cutting-edge life sciences R&D: each new researcher has to start from scratch.

We want robust experimental analysis of the component parts, but to not have to do the same studies every time. We want to build from those things that are working. There have been some great publications coming out of the lab of Katy Rezvani at MD Anderson, including some showing the clinical promise of CD19-targeted CAR NK cells. While the responses are perhaps not as persistent as you would like to see in an ideal world, they certainly represent a great starting point for others to build upon. And yet, we haven't seen the same approach being replicated in other labs, which I find surprising. It seems as though everybody is trying to go out and build their own approach, but why are we not starting from the same position? Why are we not publishing and sharing best practices?

A further issue stems from the fact that the NK field actually comprises two broad approaches: primary NK cells (which are largely akin to CAR-T and to me, make sense in the short- to medium-term) and iPSC-NK cells. The two get conflated but in reality, they are fundamentally different fields with very different roadmaps. A strategy to invest in and develop a company utilizing one approach will be quite different to a company pursuing the other. I don't believe we should even compare the two. The difficult long-term investment that will be required to answer difficult scientific questions around iPSCs mean that is likely to be a medium- to long-term solution. While I believe that the iPSC approach will eventually be an effective strategy, it is to some degree a distraction from a strategy that can serve patient needs in clinical trials—and potentially in the commercial setting—in the much nearer-term. Namely, consistent cell products derived from primary NK cells that expand well and have functional CARs.

It is important to note that CAR-NK cells are not the same as CAR-T cells. CAR-NK cell therapy development is too often seen through the lens of CAR-T cell therapy. NK cells don't function in the body like T cells, and neither should we expect a NK cell therapy to act like a T cell therapy. Clinical data seems to indicate that NK cells are fundamentally safe whereas CAR-T cells can be hazardous. CAR-T cells certainly can work, but with the wrong CAR or the wrong target, they can cause severe toxicities. With CAR-NK cells, the safety concerns are greatly reduced, which means that we can go to higher doses. We can be more aggressive in the way we design our preclinical or clinical studies. And we can re-dose.

If we can identify how to optimally freeze a CAR-NK cell product (not as great an issue as was previously supposed, in my opinion) and if we can figure out how to manufacture a consistent product with the right CAR and create a stockpile, we can approach CAR-NK cell therapy as being a much more typical, straightforward medicine than CAR-T therapy. In future, having multiple different off-the-shelf CAR-NK cell therapies available may allow us to combine them in much the same way as chemotherapies are combined today.

There are further questions on the clinical side: should we be lymphodepleting CAR-NK therapy patients in the same way that we do CAR-T cell therapy patients pre-treatment? That is an open question, currently. There is likely an optimal type of lymphodepletion for NK cells that is not the same as what is done with CAR-T cell therapy, and in fact, it may be actually make most sense to avoid lymphodepletion altogether with CAR-NK cells. We also need to understand how we can combine CAR-NK cells with other biologic modalities. For example, how can we combine a CAR-NK therapy with an antibody therapy that is going to engage with NK cells? There are opportunities to reactivate a more normal immune function by introducing healthy NK cells, potentially reactivating responses to monoclonal antibodies in patients that have become refractory. Looking further ahead, questions of therapy sequencing will also need to be addressed.

One area that perhaps does not require further research is the structure of the CAR itself. Having screened many different CARs to find ones that work in CAR-T, I would suggest that it is less important to focus on the exact CAR molecule than the techniques by which we expand and manipulate the NK cells, for instance. There are many CARs available that we already know work well—the CD19 CARs and BCMA CARs, for example. The general findings from our own research have been that those good CAR-T receptors do also work well in NK cells. So, we need not focus so intently on the specifics of the CAR molecule, or on the *in vitro* test that tells us that one CAR kills slightly more than another in an NK cell therapy. Our experience is that in reality, *in vitro* killing matters very little in comparison to both the quality and the quantity of cells that can be manufactured. Enhancing our ability to isolate, manipulate, expand, proliferate, create, freeze—in other words, to create these cellular products—is much more important than the exact nature of the CAR being added to them.

In fact, sometimes an inferior CAR *in vitro* proves to be a better CAR *in vivo*. We can't always predict how well a CAR is going to function based on *in vitro* assay. That is just as true for NK cells as it is for T cells. Having too great a focus on just exactly what the molecule is may actually be detracting from the development of the field. I believe we should just take CARs that work, and concentrate on developing better strategies for manufacturing CAR-NK therapies.

HOPE FOR THE FUTURE

Despite these challenges that face the NK cell therapy field, I remain convinced that CAR-NK cell therapy is an area brimming with the potential to become what we dreamt CAR-T could be—to have a shelf in a hospital freezer that is full of different cell therapies ready to go when patients they need them. CAR-T works well in the hematological malignancies space, but it is still a short-term solution. I believe CAR-NK can be the long-term answer.

The beauty of CAR-NK is that we can come up with generalized strategies to manufacture really compelling, high efficacy, safe products, which we can then pick and choose between, depending on the specific needs of an individual patient. In the same way that we use chemotherapy, if one treatment doesn't work, then we could have other options right there ready to go. I believe that is a realistic goal we can all build towards. The first step is to ask, 'how are we manufacturing these products?'

I'm still excited about NK cell therapies, and I hope everybody else is, too!

BIOGRAPHY

SCOTT MCCOMB is a researcher with the National Research Council Canada (NRC) Cancer Immunology team, Ottawa, ON, Canada, working to develop the next generation of CAR-T cell and other immunotherapies for cancer. Before moving to the NRC, McComb received his PhD from the University of Ottawa, Ottawa, ON, Canada followed by postdoctoral studies on cell death pathways in pediatric acute lymphoblastic leukemia in Zurich, Switzerland. McComb works with a multi-disciplinary team at the NRC to design new CAR molecules based on the unique properties of antibodies derived from llamas (known as nanobodies), testing the functionality of these nanobody-CARs for reprogramming human T cells or natural killer (NK) cells to kill cancer cells in the dish and in mouse models. McComb led the Canadian team that has developed a new highly active CAR targeting human CD22 for the treatment of lymphoma and leukemia, planned to enter clinical trial in Canada in 2024. In the long term, McComb aims to create an open toolbox of CAR parts that can be used by clinicians and researchers across Canada to build the next generation of safe, effective, affordable, and accessible cell therapies.

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CELL & GENE THERAPY INSIGHTS

INNOVATION IN CELLULAR IMMUNOTHERAPY:
HOW TO REACH MORE PATIENTS?

SPOTLIGHT

REGULATORY PERSPECTIVE

Navigating the challenges of developing delivery devices for cell and gene therapy products in Europe

Eamonn McGowran and Patrick J Ginty

The ever-increasing diversity, complexity, and potential therapeutic utility of cell and gene therapies (advanced therapy medicinal products [ATMPs]) is creating new challenges and opportunities in the field of drug delivery, whereby the ATMPs are targeting anatomical locations that may not lend themselves to systemic routes of administration. The rationale for more targeted delivery includes the potential for achieving both enhanced efficacy and safety (fewer/lower doses) for the ultimate benefit of patients. In turn, this could have a positive impact on the commercialization of ATMPs, given the relatively high cost of development, manufacture, and subsequent pricing of the products that have been approved thus far. This article aims to describe some of the challenges associated with the development of medical devices for the delivery of ATMPs, with particular emphasis on the evolving regulatory landscape in the EU and the technical challenges of delivering of ATMPs to challenging anatomical locations, such as the central nervous system (CNS).

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The number, diversity, and complexity of cell and gene therapies (advanced therapy medicinal products [ATMPs]) currently in development across a wide range of therapeutic areas is giving increasing hope to patients [1]. One of the consequences of this growing

diversity, complexity, and ever-increasing potential for therapeutic utility, is the requirement to achieve the successful delivery of these novel therapies to challenging anatomical locations in a safe and more efficient manner. The dual aim, therefore, is to develop technologies and delivery methods that enhance the safety profile of ATMPs (by minimizing off-target effects and immunotoxicity, for example) and improve the chances of achieving more durable efficacy with fewer/lower doses [2–4]. Furthermore, the latter could also potentially have a positive impact on the commercialization of ATMPs, given the high cost of development, manufacture, and the subsequent high pricing of approved ATMPs [5].

The challenges of enhancing the safety and efficacy of ATMPs through more efficient delivery to the desired target can broadly be divided into three categories; firstly, engineering or modification of the product; secondly, modification of the microenvironment into which the product is administered; and thirdly, the drug delivery system used to administer the product [6]. The first category is largely aimed at reducing the impact of the route or method of administration by engineering a product to home in on a specific target (e.g., the use of capsid engineering and/or tissue-specific promoters in AAV gene therapy). The second category is focused on creating an environment in which the product can thrive (e.g., the use of lymphocyte depletion prior to CAR-T therapy [7]) or combination therapies to enhance cellular transduction *in vivo* [8]. The third category involves the use of a drug delivery system (e.g., a medical device) that either separately or when combined with or as part of the drug product, works in harmony with the route of administration to allow specific anatomical locations to be targeted. The reality is that the success of the industry will likely depend upon progression in all three of the above categories, as no single approach will support the vast array of technologies and clinical needs. It should also be noted that the three categories are not mutually exclusive (e.g., there might be a robust case for co-development of both a delivery system and the engineering/modification of the product/product environment). However, this article is focused on the third category and how medical devices can enhance the delivery and targeting of ATMPs (whether developed separately or when combined into the same product) and how the introduction of the EU Medical Device Regulation (EU MDR) may impact this moving forward.

At time of writing, approximately two-thirds of the approved ATMPs in Europe (not including those that have been withdrawn) are delivered systemically by intravenous infusion (e.g., products that target hematological tumors, the treatment of bleeding disorders, etc. [9]). For these products, a significant change in standard clinical practice has not been required (e.g., IV based cell transplantation/blood transfusion has been commonplace for decades) and can therefore rely on much of the existing 'off-the shelf' device technology. Other routes of administration have been successful, such as retinal [10], and intraputaminal [11] but the more 'hard-to-reach' tissues and organs such as central areas of the brain (e.g., the thalamus), the lungs, kidneys, and heart may require a different approach, especially in the absence of products or product environments that have been modified to negate such issues. Furthermore, in addition to targeting the correct cells or tissues, matching a delivery technology to a medicine/clinical indication must take into consideration the pharmaceutical form and mode of action of the medicine—for example, a cell suspension or tissue engineered product that needs time to engraft into a very specific area of host tissue to achieve its function. This is still challenging in localized, immune-privileged locations such as the eye [12]. However, this presents a very complex challenge in highly sensitive, vascularized locations such as the heart [13].

As previously mentioned, the use of off-the-shelf medical devices for systemic delivery

methods is less of a concern, as many methods of IV infusion are well established as being fit for purpose and compatible with ATMPs (e.g., human cells). This is evidenced by the systemically delivered therapies that have been approved. However, for any company going down the road of highly targeted delivery, the question is not only "how will the product and clinical studies be designed to bring about therapeutic effects?" but "how do we deliver it to where the therapeutic effect will be maximized in a safe manner?" For any product, there are limitations with what can be achieved with formulation technology, so there is increasing demand for methods of administration that require medical devices that are truly fit for purpose.

Therefore, ATMP developers should consider early on in their existence, what medical devices have regulatory approval for use in the chosen indications, and if they are fit for purpose? Off the shelf devices may exist and permit early proof of concept work, and maybe early clinical trials, but the question remains: are they suitable for larger human studies and commercial use? The more difficult question is then to decide if there is a requirement to develop an existing device or develop a device from scratch. The co-development of medical devices alongside medicines and even ATMPs, specifically, is not a novel concept, companion diagnostics (IVDs) for the selection of patients in clinical studies being one such example [14]. However, the cost of medicine-plus device development is likely to be significant, especially to small companies and start-ups. One solution could be a commitment from large device manufactures to take up the challenge and work with biotech companies to develop delivery devices that will meet the need, but the reality is that there needs to be a significant market incentive (broad application) to do so. So, there is also the challenge of designing devices that are so specialized that they can only be used in very niche applications but can still be commercially viable. The ideal scenario is a device that delivers to a range of anatomical locations (or multiple locations within a complex organ such as the brain) without compromising on the choice of modality.

GENE THERAPY DELIVERY FOR CNS APPLICATIONS: A CASE STUDY

In the field of neurodegenerative and neurological disorders, there have many different approaches to the delivery of gene therapies directly to the brain or cerebrospinal fluid (CSF), ranging from intraputaminal, intra-thecal (via cisterna magna or lumbar injection), and intra-cerebroventricular. These administration routes are used for a variety of clinical indications, that may each require different targeting within tissues within the brain/CSF to either express or silence specific genes (e.g., Huntington's disease, amyotrophic lateral sclerosis [ALS], Parkinson's disease, frontal temporal dementia [FTD], etc. [15]). The use of stereotactic techniques when delivering therapies to the brain utilizes existing imaging technologies but there are still challenges with the physical delivery of ATMPs to areas deep within the parenchyma and beyond. Therefore, specialized catheters and navigation systems have been co-developed to meet this challenge [16], which encompasses the need for delivery systems that are sympathetic to the surgical infrastructure, the needs of the surgeon, and the labile nature of the drug product. This last point is particularly key, given that multiple drug modalities and formulations may need to be administered using convection enhanced delivery (CED) techniques [17]. CED is a technique that uses a pressure gradient to deliver therapeutics to the interstitial areas of the brain and CNS. It is therefore highly sensitive to backflow and air bubbles and hence, uses very slow flow rates (0.1–10 µm per minute) to deliver small volumes of drug product. This often results in long surgeries (4–10 hours) and consequently, the drug product and the device must be highly compatible to ensure

no significant loss of activity or damage due to sheer forces.

The use of the Clearpoint Neuro delivery products such as their approved navigation platform and SmartFlow® cannula has proven successful in direct brain administration and is commonly used for gene therapy and biologics delivery, including the administration of an approved product to the putamen (Upstaza, PTC Therapeutics). (The SmartFlow Cannula has 510(k) clearance from the FDA for use in the USA for the aspiration of cerebrospinal fluid or injection of the chemotherapy drug cytarabine into the ventricles. It has also been CE marked to deliver approved fluids into the brain and for aspiration of cerebrospinal fluid.) However, the use of off the shelf devices, no matter how effective, may not always be the most suitable way to deliver to the brain and CNS. For example, the use of sub-pial injections (into the membrane around the spinal cord), when compared to intrathecal or intraventricular methods, may have significant benefits in terms of targeting the cell types in the spinal motor cord required for the treatment of diseases such as the SOD1 genetic form of ALS [18,19]. AviadoBio, a pioneering gene therapy company based in the UK, has licensed a sub-pial delivery technology for its pipeline of AAV9 gene therapy products, with a view to reducing the titers of vector required to generate a therapeutic effect and minimizing potential safety concerns associated with off-target exposure [20].

Although a small sample size, there is increasing evidence that drug developers in the early stages of ATMP development are considering the importance of the administration device, either through the licensing of existing approved or cleared delivery systems [21], or the co-development of delivery systems that are yet to be approved or cleared for use. However, the challenge of gaining regulatory approval for devices or combination products in an ever-changing EU regulatory landscape remains.

THE REGULATORY CHALLENGE IN THE EUROPEAN UNION (EU)

In developing an ATMP that requires a medical device or device component for delivery, developers/manufacturers need to be cognizant of the complex regulatory position in Europe. The regulation of ATMPs and medical devices in the EU has been described in detail elsewhere [22–29]. Briefly, ATMPs fall under the regulatory framework of a medicinal biological product, principally Directive 2001/83/EC [30]. In addition, a specific legal framework for ATMPs was established by the European Commission (Regulation EC No. 1394/2007) covering evaluation, authorization, and post-authorization follow-up for ATMPs [31,32].

As outlined earlier, an ATMP may be co-developed and/or co-packaged with a device component that is required for the delivery of the medicine such as prefilled syringes, catheters, cranial implants, ocular delivery systems, and even infusion pump systems. The regulations and supportive legislation also recognize that some ATMPs may contain one or more medical devices as an integral part of the medicine, which are referred to as combined ATMPs (cATMP). These include examples such as cells embedded in a scaffold, matrices, and encapsulation systems for cells such as microspheres, among others.

For devices, it is important to be aware of the EU Medical Device Regulation 2017/745 (EUMDR) [33] and to be cognizant of the ISO standards such as ISO 13485—Quality [34] and ISO 14971—Risk Management [35]. The EU MDR creates a challenging Conformitè Europëenne (CE) marking process to ensure that safe and effective medical device are released onto the EU market. Medical devices are classified per Annex IX of the MDR, placing devices into one of four classes relative to the device characteristics and intended application and risk: class I, IIa, IIb, and III, with low-risk devices falling in class I and the highest risk into class III.

The EU MDR has also introduced updated definitions for integral devices and devices that are co-packaged with medicinal products, which they categorized as integral or non-integral as follows:

- Integral: the medical device and medicinal product form one single integrated product;
- Non-integral: co-packaged or obtained separately. The medical device and medicinal product are separate items packed together in the same secondary packaging, or the product information of the medicinal product refers to a specific device to be used and the device is obtained separately.

Thus, a developer may have a device that forms an integral product with a medicinal product or includes a medical device in the secondary packaging of the marketed medicinal product. Where a medical device is co-packaged with a medicinal product or when the product information of the medicinal product refers to a specific device to be used and the device is obtained separately, the administration device is governed by the medical device framework. These administration devices must meet the requirements of the EU MDR and will need to be CE marked.

It is important to emphasize that an ATMP may have one or more integral medical devices or active implantable medical devices components that are in scope for the EU MDR (e.g., a single integral product, intended exclusively for use in the given combination, not reusable such as a viral vector in prefilled syringe). Equally of note is that an ATMP with a non-integral device (i.e., a co-packaged, referenced device) is not a combined ATMP [24]. Basically, if the medicinal product and administration device are marketed as a single integral product intended exclusively for use in the given combination and that is not reusable, the product is governed by the medicinal products framework.

Therefore, it is of utmost importance that the product and its classification is defined as early as possible in the development cycle [27] and if it involves a device for administration, for its mode of delivery to be established. The classification of an ATMP is a vital step towards establishing the regulatory framework that is applicable and that directs the clinical development program [36]. ATMP developers have the possibility to ask the European Medicine Agency (EMA) for a scientific recommendation on the classification of the product and applicability of a product being a cATMP [37] via the Committee for Advanced Therapies (CAT), thus allowing for the determination of both the regulatory framework and the guidance recommendations to be considered.

CLINICAL TRIALS

It is clear that setting up and carrying out a clinical trial involving an ATMP is difficult, with many processes to be followed, and 'one size fits all' [22] approach cannot be applied. Numerous hurdles are to be overcome as outlined in a recent survey [38] of Europe-based ATMP developers, identifying challenges faced in the following areas: regulatory, technical, scientific, financial, clinical, human resource management, and others (including intellectual property and public perception). One such difficulty faced is identifying the relevant regulation that applies to a product. For those developing ATMPs, whilst their marketing authorization application (MAA) is overseen by the EMA, for a clinical trial, these products are overseen by the respective competent authority in the country where the trial is being run. Similarly, if there is a device component in that trial, this is also overseen by the competent authority within the jurisdiction. Each of the respective competent authorities can have a differing approach to how the device element is regulated and whether a separate device trial may be required. So, it is important this is clarified as early as possible. In conducting clinical trials,

consideration is required as to the make-up of the ATMP and whether the product may consist of an integral device, a non-integral device, both, or neither (e.g., if the ATMP delivery is intended to be device 'agnostic').

European regulators have issued helpful guidance for developers to navigate the complex regulatory space as outlined in *Guidelines on Good Clinical Practice Specific to Advanced Therapy Medicinal Products* [39] and the *Draft Guideline on Quality, Non-Clinical and Clinical Requirements for Investigational Advanced Therapy Medicinal Products in Clinical Trials* [40]. Further drug-device combinations guidance is provided in the *Guideline on Quality Documentation for Medicinal Products When Used with a Medical Device (EMA/CHMP/QWP/BWP/259165/201* [41]*)* and the *Questions and Answers on Implementation of the Medical Devices and* In Vitro *Diagnostic Medical Devices Regulations ((EU) 2017/745 and (EU) 2017/746* [42].

The above-mentioned DRAFT IMPD guide (now at revision two) highlights that, generally, the development of an ATMP should follow the same general principles as other medicinal products, and acknowledges that distinctive characteristics and features of ATMPs are expected to have an impact on product development. The GMP guidance advises that the IMPD should contain information on the characteristics, performance, and intended use of the device. For the device part, the general safety and performance requirements need to be addressed and sufficient information will have to be provided in the dossier by the sponsor of the clinical trial outlining how this meets the requirements of the medical device legislation. Where there is use of a non-integral device, it will only be permitted if the device has either been legally introduced to the market—for example, if it carries a valid CE mark—and its use is covered by the declared intended use. Where a device is being used outside of its intended use or it does not have a valid CE mark, then a clinical investigation will have to be considered. The application would be considered a combined trial according to both applicable legislations (device and medicinal) requiring approval of a clinical trial for the investigational ATMP as well as clinical investigation for the device component. Each trial needs to be reviewed on a case-by-case basis.

For trials conducted following both medicinal product and device legislation, significant challenges arise such as validation assessments and approval timelines with different procedural requirements. For the ATMP component, this will be submitted under the Clinical Trial Regulation (CTR) utilizing the Clinical Trials Information System (CTIS), whereas a clinical investigation for device component will require a separate application, being processed at the national level at each respective competent jurisdiction. For example, in Spain, the ATMP component will be assessed via the CTR, whilst the device investigation will need to be assessed under Device Regulations with the device-related documentation submitted utilizing the AEMPs device portal.

It is well recognized that conducting a combined trial is a challenge. An initial assessment by the European Commission as part of the COMBINE project [43] has identified that the implementation of a procedure for coordinated assessment of a clinical investigation application across member states represents a critical step in improving the system for combined studies in the EU.

TECHNICAL CONSIDERATIONS FOR ATMPs REQUIRING A DELIVERY DEVICE

The use of any medicinal product with a device should be demonstrated to be safe and effective. The developer/sponsor will have to establish the risk of the device and impact on the quality, safety, and/or efficacy of the medicinal product. Thus, at the early stages of the development program it is imperative to evaluate the disease target (cells or organs). Emphasis needs to be placed on estimating

the required doses for human use, and assessing the risks and impact on the medicinal product efficacy when using invasive surgery to insert a medical device for drug delivery.

ATMP manufacturing often follows the principles of quality-by-design and a risk-based approach. This also extends to use of a device with an ATMP [44]. The use of a device should be assessed from the very beginning of the development program with the screening and selection of the delivery device. As mentioned above, in some cases, current off the shelf devices may not be suitable for use with the target tissue, patient, or the mode of delivery for the therapeutic (or may interact negatively with the therapeutic). Other parameters to be considered are the patient itself (for example, whether the patient is an adult, a child, or even an infant) or if the patient may have cognitive issues. Importantly, innovators are also now expected to consider the voice of the patient in their development plans. All these factors are to be addressed in selecting or designing a suitable delivery system. The delivery device may be CE marked or could be an investigational device. If using a CE marked device, then confirming if it is used within its intended use is important [22]. If a device is being used outside of its approved intended purpose, it is likely an ATMP trial also becomes a clinical investigation of a medical device.

A particular technical challenge is the target location and successfully delivering the therapeutic to precise location. Recent approved examples are mostly limited to certain tissues, including targets such as the eye, liver, muscle, and the hematopoietic system [45]. There is an expanded interest in other locations including the ear [46], kidney [47], lung [48], and heart [13], with utilization of custom-designed delivery modes for these targets (e.g., adapted catheters). Regardless of anatomical location, where a device comes into contact with the therapeutic and/or the patient, reassurance regarding biocompatibility with that tissue (or possibly multiple tissues) will be required. The general principles that are applied to the biological evaluation of medical devices must be evaluated and may include characterization, testing, and review of existing data to assess the potential for an adverse biological reaction to occur as a result of exposure to materials. These principles are set out in international standard ISO 10993. What is to be emphasized is that all biocompatibility testing needs to be conducted prior to any clinical testing [49]. Developers of cATMPs will have to characterize the components and this includes matrices and scaffolds. Hence, all raw materials used for the device component have to be evaluated as early as possible in the development program. The formulation design also requires consideration. Most formulations are liquid solutions or suspensions. Thus, items such as flow parameters through the delivery systems have to be evaluated considering the rheological properties of the product. The potential impact on the therapeutic has to be established. A therapeutic delivered via a catheter using a pump system will have numerous needs [50]. For controlled delivery of precise volumes, usability engineering and human factors validation testing [51] approaches have to be employed to show residual risk is controlled to the necessary degree. The compatibility between the device and ATMP will require investigation. This is from both an in-use stability perspective, and the physical and chemical compatibility of the product with the device(s) (e.g., sorption, precipitation of active in solution, stability, etc.). Developers will need to conduct appropriate studies on extractables and leachables of the device components with the ATMP to be used. The rationale for the choice and optimization of the design and performance (such as dose-delivery performance and mechanical functionality of the device) with dose accuracy/delivered dose uniformity must be established. To this end, confirming that the medical device/devices that is/are part of the product meet the essential requirements laid down in the EU MDR and applicable harmonized standards [50,52] is a requisite. It

is vital to be cognizant of the ISO standards such as ISO 13485 (Quality) and ISO 14971 (Risk Management). The device aspect will have to be developed to meet a range of standards such as those around Software (IEC 62304), Usability Engineering (ISO 62366), Sterilization (ISO 11135; ISO 11737), Clinical investigation of medical devices (ISO 14155), and Intravascular catheters (ISO 10555), among others.

Most ATMPs are developed by micro, small, and medium-sized companies as well as universities and academia. It is widely acknowledged that these innovators are not experts [53] in design, quality management, and regulatory strategy. Bringing these modalities to successful first-in-man studies and then on to successful authorization requires collaboration with cross-functional teams and experts in not just biologicals, but also manufacturing, quality, clinical, and device development. Difficulties can arise at the intersection of these disciplines as, for example, biologists and engineers have differing approaches. Hence, in addition to the technical challenges to be overcome, it is also imperative to have good project structure and planning. The involvement of an experienced regulatory professional is highly recommended to navigate through the regulation and define the most-appropriate regulatory strategy.

It must be emphasized that developers should seek early advice at both the national or European level to guide product development, and this includes aspects of the device component of any ATMP. As well as the previously mentioned classification system, the EMA offers a range of advisory services and incentives to support the development of ATMPs through scientific advice and protocol assistance; orphan designation; the Innovation Task Force; The PRIORITY MEDICINES scheme (PRIME), the micro, small, and medium-sized enterprise (SME) office, and certification of quality and non-clinical data for SMEs [37]. In all these above agency interactions, the relevant aspects of the device should also be presented to make the agency aware of the device components.

INVOLVEMENT OF NOTIFIED BODIES (NB) IN MARKETING APPLICATIONS

In the context of the marketing authorization, a commentary on article 117 of the EU MDR amendment to Directive 2001/83/EC is important. Where a medicinal product incorporates a medical device, or a medical device is integral to the medicinal product and used for its administration, then a general requirement for a NB opinion is needed. In both the EU available guidance for drug-device combinations (*Guideline on Quality Documentation for Medicinal Products When Used with a Medical Device (EMA/CHMP/QWP/BWP/259165/201*) [41] and the *Questions and Answers on Implementation of the Medical Devices and* In Vitro *Diagnostic Medical Devices* regulations [42]), it is clearly stated that in the case of cATMPs, Article 117 does not apply. Where medical devices are co-packaged with ATMPs or separately obtained devices and that are referenced in the medicinal product's product information, the content of the above guidance should be taken into consideration. However, this does not mean that the requirement for engagement and assessment of medical device by a NB as part of an ATMP approval is not needed [54]. Article 9 of Regulation (EC) No 1394/2007 on ATMP refers to the involvement of NB and the 'Procedural advice on the consultation of NB in accordance with Article 9 of Regulation (EC) No. 1394/2007' (EMA/354785/2010) [55] outlines the involvement of a NB in the evaluation of cATMP. The result of the evaluation of the medical device component by a NB shall be included in the MAA and recognized. If no NB assessment is available at time of MAA, then the EMA/CAT may seek an opinion on the conformity of the device part with the essential requirements of the relevant Medical Device Regulation. For a cATMP,

any procedural interactions will involve all parties (i.e., the EMA/CAT, the NB, and the applicant for the MAA). For any ATMP developer the potential for a NB involvement will have to factored in as part of their development strategies.

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CELL & GENE THERAPY INSIGHTS

INNOVATION IN CELLULAR IMMUNOTHERAPY: HOW TO REACH MORE PATIENTS?

SPOTLIGHT

Cellular immunotherapy—a matter of delivery?

Nina Bauer
SmartCella

"To reach more patients with cellular immunotherapy—or really, with any of the novel modalities we are currently developing—we have to prove that they can become mainstream, both in their delivery and their affordability."

VIEWPOINT

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INTRODUCTION

The field of cellular immunotherapy is ever expanding. What started in 2017 with autologous CAR-Ts to treat liquid tumors has now expanded to successfully treating autoimmune disorders such as Progressive Multiple Sclerosis [1], and most recently, systemic lupus erythematosus, idiopathic inflammatory myositis, and systemic sclerosis [2].

Contrary to cancer, autoimmune therapies have seen some indication that off-the-shelf allogeneic approaches might be promising. If this can be confirmed, more patients will be able to receive treatments that are more cost-effective and easier to deliver.

We cannot, however, ignore the fact that in large segments of the solid tumor space, cellular immunotherapies have been unsuccessful. Casual conversation with industry colleagues as well as literature research implicate the tumor microenvironment (TME) in preventing CAR-Ts from penetrating and eradicating the tumors, amongst other factors. The cells get 'frozen out', I've been told. Consequently, the field has expanded from T-lymphocytes to cell types that have much more pronounced tissue penetration properties, including tumor infiltrating lymphocytes and natural killer cells, with two recent approvals: AMTAGVI™ by Iovance and TECELRA® by Adaptimmune.

If getting into the tumor is one of the key hurdles, this begs the question of whether injecting cellular immunotherapies directly into tumors could be a way to overcome this?

This idea is, of course, not a novel one. Big pharma has spent significant time and investment on intratumoral therapeutic development, with targeted radiotherapy and immunotherapies featuring prominently. Notably, these efforts have largely been abandoned (Table 1), despite some positive outcomes. Remaining players are focusing on more novel modalities such as oncolytic viruses, and quite a few are developing accompanying devices to facilitate delivery into the tumor. While the reasons for big pharma's departure from the space are difficult to discern, anecdotal reports point to limited success in routine administration into tumors, i.e. a procedural limitation rather than a therapeutic one. This leads me to the question: with the appropriate device, could these therapies (have) be(en) effective and successful?

With that background in mind, might CAR-Ts, natural killer cells, tumor infiltrating lymphocytes, and others also show improved efficacy if delivered directly into the tumor?

To the best of my knowledge, this has yet to be tested. Up until now, the focus has been on optimizing CAR constructs to help direct cells to their target. And it stands to reason that even if injected into the tumor parenchyma, the TME would possibly incapacitate the cells' functionality. With that said, adjuvants and neoadjvants with the capacity to precondition the TME and make it more permissible, or immune checkpoint inhibitors delivered to the tumor prior to a cellular therapy or in conjunction with one, could be an approach worth investigating.

WHEN MEDICAL DEVICES COME INTO PLAY

Leveraging medical device technology for the delivery of novel modalities is an emerging field. Vertex's VX-264, for example, is a drug

TABLE 1

Abandoned intratumoral therapeutic efforts by large pharma (Citeline Analysis, 2024).

Company name	Number of drugs	Therapeutic class
Nektar Therapeutics	1	TLR 7/8
Emergent BioSolutions	1	Immunological oligonucleotide
AstraZeneca	1	mAb + radioisotope
Bristol-Myers Squibb	2	mAb, TLR 7/8
GlaxoSmithKline	1	Immunological oligonucleotide
Merck & Co.	2	VV; immunological oligo
Merck KGaA	1	mAb
Novartis	2	Immunological oligo, ChemoTX
Sanofi	1	Viral vector
Takeda	1	mAB

device combination where a channel array protects the insulin producing cells from the body's immune system. However, it has to be surgically implanted, and it is yet unclear how long it will persist.

BioCardia, on the other hand, have developed a proprietary catheter technology (Helix™) with a screw-like tip that is threaded into the heart's chambers and releases their cell therapy directly into the cardiac muscle wall to repair damaged tissue. At SmartCella, our own cardiac cell therapy, which has been in development with AstraZeneca and is now approaching the clinic, has been shown in a pig model to functionally integrate with cardiac tissue and replace damaged cells when delivered directly into the tissue with our proprietary Extroducer® endovascular delivery device (Figure 1). This represents a minimally invasive approach to what would normally be a high-risk open-heart surgery for very fragile patients.

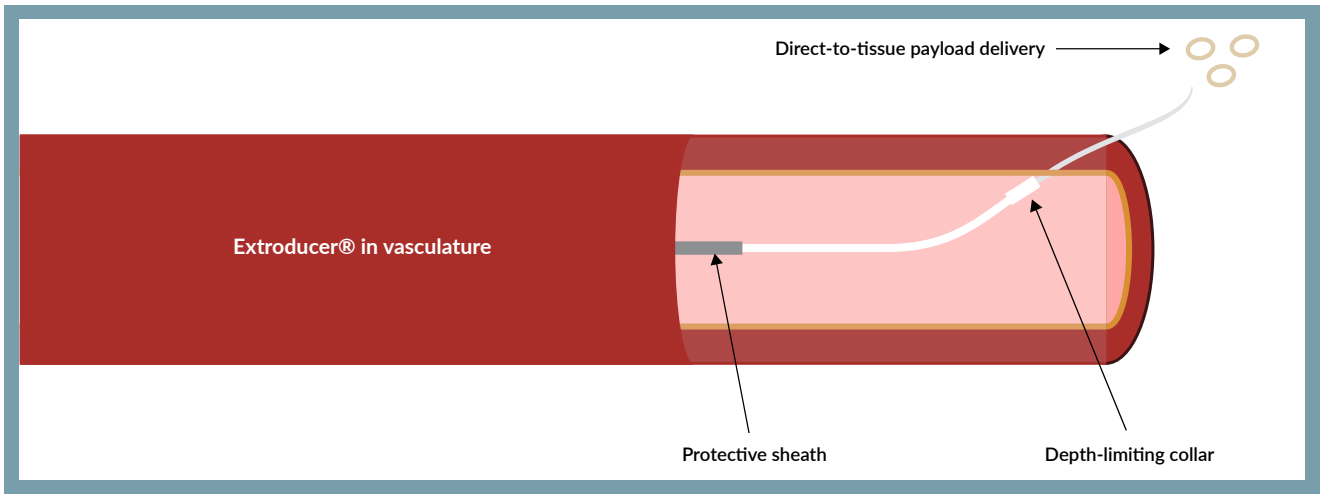
In the gene therapy space, two therapies delivering vascular endothelial growth factor (VEGF) have progressed to Phase 2 clinical studies for refractory angina. Both studies demonstrated drug safety, with the Finnish team around Professor Seppo Ylä-Herttuala providing up to eight years follow-up and showing sustained symptom relief [3–5]. Dr Ylä-Herttuala's team applied an image-guided catheter technology (NOGA® Myostar™, Johnson & Johnson) to inject their viral payload directly into the myocardium. XyloCor delivered their early trial via a surgical approach, and after observing severe adverse events related to the procedure, have recently licensed our Extroducer® technology for targeted delivery [5,6].

The attentive reader will have noticed the cardiac theme among my examples, a step away from cellular immunotherapy. Leveraging endovascular technique as a route of administration has been standard practice for decades, one example being transcatheter therapies for the heart. Interventional radiology has become a subspecialty of cardiology and hence, conducting cardiac clinical trials using catheters is nothing out of the ordinary.

In the oncology space, one of the earliest reports of endovascular treatment of tumors dates to 1904, when Dawbarn performed transcarotid paraffin and petroleum embolization of a facial sarcoma [7]. This has subsequently lead to the development of arterial chemoembolization, or TACE,

FIGURE 1

Extroducer mechanism of action.



A long, thin, flexible, needle that can be navigated through the vasculature using routine endovascular equipment; at the target site, the needle can penetrate the vessel wall and deposit payload directly in the tissue.

approaches, which today are most established in the treatment of hepatocellular carcinoma. However, outside of this niche, interventional oncology has not yet become a mainstream approach.

TARGETED DELIVERY—AN ECONOMIC ARGUMENT?

At the time of writing and echoed by some of the other articles in this issue of *Cell & Gene Therapy Insights*, the lack of efficient, robust, scalable manufacturing approaches remains a key concern when considering market supply. This is one reason for the high cost of novel modalities, including cellular immunotherapies, which still represents a major hurdle for the wider adoption of potentially curative treatments. Therefore, reducing the need for large doses could bring down the price. Delivering payloads directly to the target location would remove the need to account for systemic losses, which inevitably occur when delivering intravenously, thus requiring much lower doses. It would also reduce the risk of off-target effects (Figure 2).

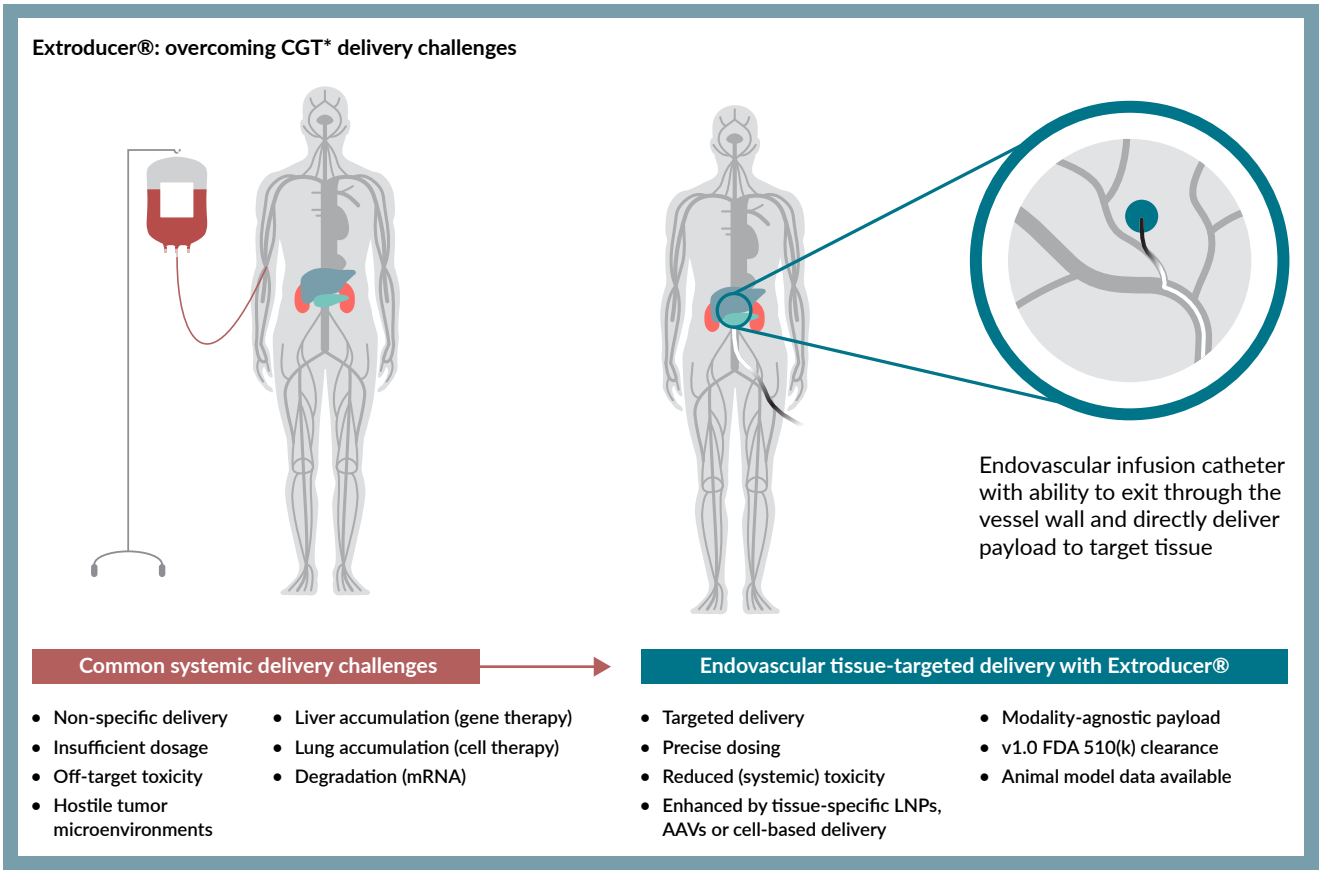
While this cost reduction might be partially eaten up by the need to involve interventionalists, reducing side effects and in some cases surgical interventions with minimally invasive approaches could result in shorter hospital stays, faster recovery, and, in some cases, remove the need for redosing.

IN SUMMARY

You may say that what I have presented in this Viewpoint are mostly hypotheses. However,

FIGURE 2

Considering targeted delivery in a novel modality context, where systemic administration often does not yield the desired results.



over the past 15 years that I have been involved in this industry, it wasn't incremental or continuous improvements that allowed for our big leaps. It was the breakthrough innovations like Carl June and Bruce Levine testing out a CD-19 CAR-T when no other treatments remained that triggered radical transformations in our space.

To reach more patients with cellular immunotherapy—or really, with any of the novel modalities we are currently developing—we have to prove that they can become mainstream, both in their delivery and their affordability. We have now shown that the basics are there, so maybe it is time to think outside the box and learn from adjacent disciplines.

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BIOGRAPHY

NINA BAUER is a trained neuroscientist. Her career quickly centered on the cell and gene therapy space, holding various technical and commercial roles at the Scottish Center for Regenerative Medicine and the Cell and Gene Therapy Catapult. She deepened her global commercial experience establishing Lonza's autologous cell therapy business and strategic positioning by incorporating the Cocoon™ technology with a vision for near-patient manufacturing, as well as leading commercial teams at MilliporeSigma/Merck KGaA focusing on the cell therapy product portfolio, gene therapy manufacturing services and CRISPR IP licensing. Aside from large corporate and public sector leadership roles, Nina established her start-up and C-suite acumen as the Chief Commercial Officer for FloDesign Sonics, leading to the company's acquisition by MilliporeSigma, as well as a wide range of advisory board engagements. Nina currently serves as the Chief Business Officer of SmartCella, Stockholm, Sweden, a Swedish biotech developing therapeutic delivery approaches and pluripotent stem cell-based therapies.

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CELL & GENE THERAPY INSIGHTS

INNOVATION IN CELLULAR IMMUNOTHERAPY: HOW TO REACH MORE PATIENTS?

SPOTLIGHT

INTERVIEW

Addressing autoimmune diseases with engineered MSCs

As the cell and gene therapy field matures, both new and old approaches are being leveraged and combined to enable the field to expand into indications beyond oncology. In this interview, **David McCall**, Senior Editor, BioInsights, and **Miguel Forte**, CEO, Kiji Therapeutics; President, ISCT, discuss a novel engineered off-the-shelf approach leveraging that old warhorse of cell therapy, mesenchymal stem cells.

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Q What are you working on right now?

MF: Since transitioning to the cell and gene field some years ago, driven by the field's scientific promise and the opportunity to help patients, I have enjoyed working with various different companies, a few of which I continue to serve as a Board Member and Advisor. Additionally, I recently co-founded a company, Kiji Therapeutics, that uses gene-engineered stem cells for optimal therapeutic delivery.

I am also very active in organizations like the International Society for Cell and Gene Therapy (ISCT), of which I am now President, and the Alliance for Regenerative Medicine (ARM), of which I am a member of the Board of Directors.

"...we are seeing fantastic developments in treatments for B-cell-based autoimmune diseases with CAR-T therapies."

Q What is your reading of the recent expansion beyond oncology in the cellular immunotherapy field?

MF: Cell therapy originally began with using cells to perform their natural functions—for example, in both blood transfusions and bone marrow transplants, cells are taken from one context and used in another to carry out their function. However, the technology has now expanded, offering us opportunities to tweak and modify cells to change their power and increase specificity.

This engineering of cells for a specific purpose is both the present and the future of cell and gene therapy. Whether this is done in the *in vivo* or *ex vivo* setting, and whether the cells are sourced from autologous or allogeneic origins, the principle is always the same: take a cell, optimize its function, and deliver a therapeutic benefit to patients. I believe that in the future, we will optimize cell sourcing, with induced pluripotent stem cells (iPSCs) being one key approach, as well as cell function and targeting through the means of gene editing, transduction, and transfection.

Another sign of maturity in the field is the expansion of technology into other indications. Oncology was the main entry point, as it so often is for biotechnology, but cell therapy technology is now expanding into the treatment of autoimmune diseases in particular. For example, we are seeing fantastic developments in treatments for B-cell-based autoimmune diseases with CAR-T therapies.

Q As CEO of an early-stage biotech in the space, what are you seeing in terms of reaction from the investor community?

MF: There is a clear sense of cautious optimism. People are acknowledging that we are no longer in the boom period of a few years ago. Investment decisions are more rational and deliberate as a consequence, but investments are still happening.

There have also been some pushbacks and concerns about cell and gene therapy as the field matures. We may not be getting everything we want, but we are getting what we need. The field is definitely in a better place than last year, although still recovering, resetting itself, and improving. Overall, while there is optimism, resilience and realism remain crucial.

Q Tell us more about Kiji's technology and pipeline—what differentiates your approach?

MF: We offer an allogeneic, off-the-shelf, easy-to-use, low-cost solution to modulate regulatory T cell (Treg) function and address autoimmune conditions primarily driven by Tregs. We engineer cells with a lentiviral vector that delivers CXCR4 and IL-10. This engineering ensures that the cells target inflammation sites (thanks to CXCR4) and release large quantities of IL-10, providing an immunoregulatory stimulus that enhances Tregs and regulatory B cells (Bregs), and also impacts the innate immune system.

We use mesenchymal stem cells (MSCs) because their safety is well established, and they are easy to develop and manufacture. We will derive our MSCs from iPSCs, combining ideal sourcing with a targeted mechanism of action that directs the cells to inflammation sites to deliver IL-10.

In our first indication, graft-versus-host disease (GvHD), we have shown that untreated animals exhibit a certain level of disease, which is partially controlled with naive (non-engineered) MSCs, and significantly better controlled with our engineered cells. This demonstrates a benefit beyond what MSCs alone can provide in GvHD, and it means that we can build on their documented benefit in this indication—for example, Mesoblast is set to have its naive MSC product approved for GvHD. We aim to be in the clinic with our enhanced approach within the next 12 months—we have a clinical study ready to go, and we are just now finalizing the preclinical data package.

Beyond GvHD, we are targeting inflammatory bowel disease (IBD). We have documented biodistribution to the site of inflammation, local IL-10 delivery, and modulation of Tregs, Bregs, and the innate immune system, leading to both an increased initial benefit and a more prolonged, sustained benefit. (The benefit actually increases upon re-challenge in animal models).

Our differentiated approach targets the gaps in autoimmune treatment not currently addressed by CAR-T cell therapies. Instead of targeting B cells, we focus on T cells with an allogeneic, practical, low-cost and off-the-shelf solution for autoimmune diseases.

Q Can you expand on the considerations with, and potential benefits of, gene-engineered MSCs versus other cell types and modalities in autoimmune disease applications?

MF: MSCs are the warhorses of cell therapy. They were the first entrants into the field but in those early days, they were used in a way that was not optimized. They have been well documented as being reasonably easy to use, easy to produce, and safe. We recognize their multiple mechanisms of action, such as those driven by CXCR4 and IL-10 and look to leverage them. Ultimately, we aim to take this useful, fundamentally safe tool, which has demonstrated some efficacy, and look to enhance the potency of that efficacy.

This brings us back to the beginning of our conversation: we take cells and optimize them for a specific function. That is exactly what we are doing with MSCs. Additionally, we are

optimizing the source by using iPSCs instead of donor cells, which is more practical from a manufacturing standpoint. The key is the enhancement of function through the transfer of genes like IL-10 or CXCR4 to ensure the cells go where they are needed.

This approach is similar to CAR-T therapy, where the CAR guides the cells to target an antigen and deliver a function. We can further enhance this with armored CARs and other engineered modifications. The principle that we are applying to MSCs remains the same: engineer the cell to achieve targeted therapeutic effects.

Both the biggest advantage and disadvantage of MSCs is their long history. We know how to use them, but people question why we are now focusing on cells that have been around for a while and have not delivered enough results in that time. Again, the reason is that they were not efficiently optimized. They were very safe and had some efficacy but were not truly differentiated. We are enhancing their benefits by gene-engineering them to deliver a localized immunomodulatory stimulus.

Our approach aligns perfectly with the current trends in cell and gene therapy. The important thing is to find the right cell, the right mechanism to enhance, and the right patient population to deliver value. As I mentioned earlier, it does not make sense to compete directly with CAR-T therapies like CD19 in B-cell-driven autoimmune diseases. Instead, we have a unique, differentiated, and very competitive approach to increasing Treg function and addressing autoimmune conditions driven by T cell dysfunction.

Q What are the considerations and potential benefits of gene-engineered MSCs from a manufacturing standpoint?

MF: As I mentioned, MSC products are easy to culture, but they are currently mostly donor-based. In fact, our first asset is actually adipose-derived and donor-based, using the same cell source as an approved MSC product in Europe.

However, we have already developed an R&D-grade product, which is an iMSC—an MSC derived from an iPSC. This will provide greater consistency, larger quantities, increased productivity, lower costs, and more opportunities for engineering. By engineering earlier in the process, we can have a cell line ready to go, thus optimizing manufacturing for consistency, productivity, cost, and ease of production.

The field has advanced not only from a clinical perspective but also in manufacturing capabilities, offering better and more cost-effective production methods. We believe we can reduce our cost of goods to very attractive levels, making our proposition both valuable to patients and crucially, sustainable within the payer system.

Sustainability is vital for the field; for investment, and, most importantly, for ensuring broad patient access. One of my main concerns, and something I will emphasize during my tenure as ISCT President, is addressing two key elements: global access for patients with advanced therapies, and the sustainability of the system from industrial, academic, scientific, and patient access perspectives—all while maintaining ethical standards, of course.

"One of my main concerns...is addressing two key elements: global access for patients with advanced therapies, and the sustainability of the system from industrial, academic, scientific, and patient access perspectives..."

Q What for you are some key next steps for the cell therapy industry and the wider community towards achieving commercial product success in the autoimmune disease space?

MF: Autoimmune diseases are the new focus and topic of the moment, which is great. However, in cell and gene therapy, we must always keep several aspects in mind. First and foremost are safety and efficacy. We have discussed some of these elements, and it always comes down to a risk-benefit discussion. This is a crucial concern we must always consider.

Secondly, there is technology development, particularly in manufacturing. It is important to improve manufacturing processes, as this will not only make production easier but also reduce costs. Combining these factors brings us back to the fact we need to focus on developing the field by enhancing the technology behind our products and documenting their benefits within a risk–benefit framework for the right patient population. By doing this, we can deliver sustained and increasing therapeutic opportunities to patients with various conditions, particularly autoimmune diseases.

Q Lastly, can you sum up one or two key goals and priorities for Kiji Therapeutics over the foreseeable future?

MF: We are currently raising funds with two clear objectives. The first is to execute a clinical study and obtain clinical data on our first asset, targeting CXCR4 and IL-10, in the treatment of GvHD. We aim to deliver this by the second half of 2025. The second objective is to develop a GMP platform of high-quality, iPSC-derived MSCs engineered for specific purposes. The initial gene will be CXCR4 and IL-10, but we are also exploring additional genes through potential academic collaborations.

So, our deliverables by the second half of 2025, beginning of 2026, will be the clinical data from our first asset and a platform of iMSCs engineered with various genes for different therapeutic purposes. If successful, we will proceed to a larger study and advance several assets into the clinic, with IBD being the next likely indication.

BIOGRAPHY

MIGUEL FORTE is the President and Board of Directors chair of the International Society of Cell and Gene Therapy (ISCT). He was previously Chief Commercialization Officer and Chair of the ISCT Commercialization Committee (2014–2020) leading the expansion of the industrial community, strengthening the Committee to better represent the industrial translational objectives and needs of society membership. He is the CEO and Co-Founder of Kiji Therapeutics, Executive Chairman of the Board of StemBond and consults through mC4Tx to several organizations. He is a Professor at the Lisbon University and Board of Directors and Executive Committee member for the Alliance for Regenerative Medicine (ARM). He was previously CEO of Bone Therapeutics (2019–2022) and CEO of Zelluna Immunotherapy (2017–2019). From 2010–2017 he was CMO/COO of TxCell, and from 2006–2010 he was VP of Global Medical Affairs at UCB. In 2004, he joined Nabi Pharmaceuticals as the VP of Medical/Regulatory Affairs for Europe, leading from inception the creation of the vaccine company in Europe. After several public sector clinical, academic, and regulatory positions in Portugal and at the European Medicines Agency (EMA), namely as a CHMP member, Dr Forte spent 6 years with BMS (1998–2003) as Country Medical Director, Executive Director, and VP of International Medical in Portugal and Belgium. He holds an MD, specializing in infectious diseases, from the Faculty of Medicine, University of Lisbon, Portugal a PhD in Immunology from the University of Birmingham, UK and a certificate on Health Technologies Economics, Stockholm School of Economics, Sweden. He is a Fellow of the Faculty of Pharmaceutical Medicine of the RCP in the UK.

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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT

INTERVIEW

Analyzing early successes, current challenges, and future opportunities in the *in vivo* cellular immunotherapy field

In vivo cellular immunotherapy has captured the imagination of many due to its obvious potential to alleviate the current supply chain cost and complexity issues associated with CAR-T cell therapy. **David McCall**, Senior Editor, BioInsights speaks to **Ye Zeng**, Postdoctoral Fellow, Bioengineering, Mitchell Lab, Department of Bioengineering, University of Pennsylvania, about the current state-of-the-art in this area of research, and the Mitchell Lab's own work to develop non-viral delivery platforms with the capabilities to drive the field forward into a variety of therapeutic areas and disease indications.

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Q What are you working on right now?

YZ: We are currently working on two projects at the Mitchell Lab. The first involves developing a potent next-generation LNP for immunotherapeutic cancer vaccines while incorporating a novel adjuvant to stimulate immune response. The second project focuses on developing other LNPs for specific targeted delivery.

Q The Mitchell Lab, and UPenn in general, are at the forefront of efforts to unlock the potential of ***in vivo*** CAR-T and other engineered cellular immunotherapy modalities. What is your analysis of the current status of R&D in the field?

YZ: CAR-T therapy has already gained significant success in treating B-cell leukemia, but we are now focused on applying this technology to a broader range of diseases.

More specifically, we are trying to improve the targeting and efficacy of CAR-T cell therapy for solid tumors—for example, by incorporating the HER2 protein in breast cancer. In addition, we are researching dual or even multi-targeting of CAR-T cells.

We are also working on non-viral delivery systems for CAR-T cell therapies. Nowadays, we have more choices for biotherapeutic delivery systems such as LNPs, which have been proven successful in the mRNA-based COVID-19 vaccines. We aim to develop a large library of LNPs, which could be used to deliver CAR-T cells—or indeed, cells engineered with other antigen receptors—for *in vivo* cellular immunotherapy.

Another important goal of current CAR-T cell therapy R&D is to reduce toxicity. Some CAR-T therapy patients experience cytokine release syndrome or neurotoxicity, for example, and the aim is to reduce these negative effects. One of our former colleagues, Ningqiang Gong, recently produced a research paper published in Nature Materials about reducing cytokine release syndrome by attaching a hydrophilic polymer polyethylene glycol to the surface of CAR-T cells, which proved to be successful [1]. Our ultimate goal at the Mitchell Lab is to develop a universal CAR-T with reduced toxicity, but there are many obstacles to be negotiated before that goal can be achieved.

Q There is little doubt that an ***in vivo*** solution would represent the 'holy grail' for cellular immunotherapy—but what's your assessment of the remaining barriers to success in the space?

YZ: Firstly, there are many hurdles associated with specific *in vivo* delivery due to the complexities of the human body. In cancer treatment especially, there are many complex challenges, including blood-tissue barriers, rapid immune system clearance, and a limited ability to penetrate solid tumors.

Another challenge is ensuring that once the nanoparticles, engineered cells, or other therapeutics have been delivered to the body, they are efficient in reaching the target, and also have sufficiently sustained expression to achieve therapeutic outcomes.

Finally, there are safety and immunogenicity challenges, of course. Every person may have a different reaction to immunotherapy, therefore, ensuring a safe immune response to whichever vector you are using is crucial.

"Combining LNPs with CRISPR-Cas9 gene editing tools to correct mutant genes is a further area of interest for us. This approach could be particularly effective for treating autoimmune or single-gene defect disorders, as it would allow for more precise and easier gene editing."

Q Can you expand on the Mitchell Lab's efforts to overcome these issues, and on the particular delivery platforms and payloads that are showing promise in your studies?

YZ: Some of these hurdles could be overcome by developing next-generation non-viral delivery vehicles, which is something our team is working on as I've mentioned. One area of focus there is developing a 'naked' LNP—one without any modifications—that is potent enough to enter the T cells or other cells of interest. Some PhD students at the Mitchell Lab are also working on developing naked LNPs to deliver mRNAs for autoimmune disease treatment. Antibodies could also be attached to LNPs, which could facilitate targeted delivery to specific cells inside the body.

Combining LNPs with CRISPR-Cas9 gene editing tools to correct mutant genes is a further area of interest for us. This approach could be particularly effective for treating autoimmune or single-gene defect disorders, as it would allow for more precise and easier gene editing.

Finally, some other researchers in the Mitchell Lab are working on combination therapy of CAR-T cells with conventional mRNA-based cancer vaccines, which could broaden the therapeutic choices available for a variety of different indications.

Q Which emerging or established R&D analytical tools are proving most valuable in providing the insights needed to progress the *in vivo* cellular immunotherapy field?

YZ: One of the key tools used in our laboratory is barcoding technology. When we inject the nanoparticle into the body, we can use this sequencing technology to identify which specific subpopulation of cells will be targeted or transfected. This in turn allows us to gain more information about the biodistribution of the nanoparticles. For example, we can determine if the nanoparticles target specific cells, such as endothelial or macrophage cells in the liver. This understanding can help us develop targeted therapies for diseases caused by endothelial or fibroblast dysfunction. In essence, precise biodistribution analysis provides valuable insights relating to the specific delivery of nanoparticles and cellular immunotherapy.

We are also exploring advancements in next-generation RNA-based modalities, such as circular RNA and self-amplifying RNA (saRNA) to improve therapeutic safety and efficacy. Using these forms of RNA can reduce the amounts of nanoparticles or nucleic acids required to deliver into cells, lowering side effects and toxicity.

Q What will be some key next steps for innovation regarding the enabling toolkit, for you? What is on your wish list?

YZ: I don't know how realistic a wish this might be, but having real-time *in vivo* monitoring equipment would allow us to track nanoparticles throughout the entire body. For example, this technology could be integrated with radiology or MRI scans to allow us to monitor the behavior of engineered cells or LNPs. Such an *in vivo* monitoring solution would provide a lot of useful information that could help us unravel the biological complexities of our bodies.

Q Can you share your vision for the future applications of ***in vivo*** cellular immunotherapy?

YZ: As scientists, we aim to develop treatments for a wide range of diseases beyond the successful CAR-T therapies for blood cancer, which have already seen huge success.

Firstly, based on the knowledge gained from developing cell therapy for B-cell leukemia, we could potentially extend these treatments to solid tumors. Beyond that, there are infectious diseases such as those caused by COVID-19 and HIV. Here, we are already seeing clinical trials using CAR-T cells to target the HIV glycoprotein, GP120, and these pioneering studies could provide valuable insights.

As we have already discussed *in vivo* cellular immunotherapy could be applied to autoimmune diseases, such as type 1 diabetes, rheumatoid arthritis, and multiple sclerosis. There are currently some pilot clinical trials underway testing CAR-T cells for the treatment of systemic lupus erythematosus, which may help us understand how to apply *in vivo* cellular immunotherapies more broadly for autoimmune diseases.

Furthermore, this field could be extended to the treatment of genetic disorders, potentially by harnessing emerging gene editing tools like base editing and prime editing to correct mutated genes.

Last but certainly not least, cardiovascular disease treatment could benefit from these advances in cellular immunotherapy. According to a paper published in Science in 2022, CD5-targeted LNPs for T cell transfection *in vivo* could be used to treat cardiac failure [2].

Q Finally, can you share one or two key goals or priorities for the Mitchell Lab over the foreseeable future?

YZ: Nucleic acids carry great potential for advancing treatments in many therapeutic areas, as well as enhancing regenerative medicine, immunotherapy, and gene editing. Our key goal is therefore to develop potent novel biomaterials and nanoparticles in the context of nucleic acid delivery, including mRNA, saRNA, miRNA, and CRISPR-Cas9, which could be applied in the treatment of multiple diseases. Additionally, we will continue developing next-generation targeted nanoparticles and other delivery systems that can work in combination with engineered cell therapy products to achieve better therapeutic outcomes for patients.

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BIOGRAPHY

YE ZENG earned her MSc in Pharmaceuticals from Sichuan University, Chengdu, Sichuan, China in 2016. She completed her PhD at Leiden University, Leiden, Netherlands in 2022, working in the Supramolecular & Biomaterials Chemistry group under the supervision of Professor Dr Alexander Kros. Her doctoral research focussed on the development of mRNA and drug delivery systems using lipid-based nanoparticles. Following her PhD, she spent a year conducting postdoctoral research at the Leiden Institute of Chemistry, focusing on RNA therapeutics delivery, supported by an NWO-XS grant. In January 2024, she joined Professor Dr Michael Mitchell's group at the University of Pennsylvania, Philadelphia, PA, USA, as a postdoctoral researcher, where she primarily focuses on mRNA vaccines for cancer immunotherapy.

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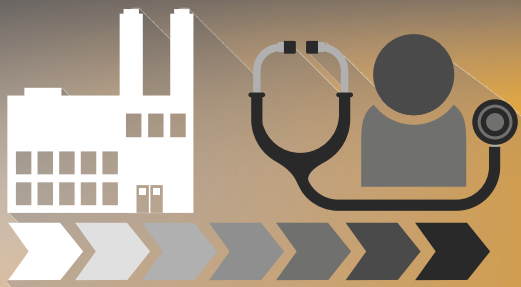
CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN CHANNEL EDITION

Supply chain digitization



AUGUST 2024

Volume 10, Issue 7

CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN DIGITIZATION

CHANNEL CONTENT

INTERVIEW

Streamlining cell and gene therapy supply chains: using semi-automation and AI for cost reduction

Abi Pinchbeck, Editor, *Cell & Gene Therapy Insights*, speaks to **Stephan Kadauke**, Associate Professor of Clinical Pathology and Lab Medicine, Children's Hospital of Philadelphia, exploring the complexities of traditional supply chains in cell and gene therapies (CGTs) and how semi-automated processes could help to simplify them. They also discuss how artificial intelligence (AI) and machine learning could be used to automate and reduce the costs of CAR-T manufacturing.

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Q First and foremost, what are you working on right now?

SK: I am currently an Associate Professor in the Pathology and Laboratory Medicine department at the Children's Hospital of Philadelphia (CHOP). I serve as the Associate Director of the Cell Based Therapy Lab, which is a GMP facility that manufactures CAR-T cells and other types of cell therapies. And I'm also the Medical Director of the CGT Informatics group.

I am particularly excited about finding ways to automate and reduce the cost of CGTs. Recently, there have been some fantastic advances in CAR-T cell therapy products for diseases

in great need of treatments, as well as other cell therapies being approved by the US FDA. Unfortunately, some of these therapies are incredibly expensive, which limits access to patients. However, we have figured out how to use semi-automated processing systems to decrease the logistics required to manufacture CAR-T cells, and we hope that this is a step towards ultimately reducing the costs of these therapies. Our experimental protocol was approved by the FDA and we are now actively treating patients, which is exciting.

The manufacturing cost is much lower than the cost of a commercial product. We are actively working on expanding this paradigm to other kinds of therapies, which is also something I am excited about.

Q What are the key challenges that CGT specifically presents to supply chain management?

SK: When considering the supply chain, there are huge challenges associated with the traditional CGT manufacturing process that could be solved with a semi-automated process, consequently reducing the cost.

To provide more context, manufacturing CAR-T products starts with cells from a patient's apheresis collection. T cells are then selected, purified, and genetically modified. Afterward, those T cells are transduced with a lentiviral vector, and grown to achieve at least one therapeutic dose. In the traditional manufacturing process, all of this is done manually and involves using different machines, types of bags, and reactors. Each one of these components often has its own supply chain, which usually requires contracts with different vendors.

One of the challenges of traditional cell manufacturing processes is that supply chains are complex and require quality agreements and relationships with various vendors. One of the advantages of buying into the ecosystem of a vendor of a semi-automated cell processing system is that this can simplify things.

Another relevant challenge regarding supply chains is expanding patient access outside of the US. For example, we have an initiative with the National Cancer Institute of Brazil in Rio de Janeiro, where there are concerns about building up these supply chains as they simply do not yet exist. Our colleagues in Brazil are interested in building up lean supply chains that are robust and less complicated.

Q As the Medical Director of Cell and Gene Therapy Informatics, can you detail the novel informatics tools and technologies being applied in the space?

SK: At CHOP, we have a unique informatics team dedicated to our CGT program. One of the things I'm excited about in CGT is using AI to look up information more effortlessly. For example, we have many standard operating procedures (SOPs) and regulations to consult for specific technical questions. For example, when we are dealing with a bone marrow product, there might be questions regarding the expiration date. The answer to this question is buried somewhere in our SOPs and it might take some time to look up. This is a tedious and time-consuming process that might be costly and pose risks–inspections by the FDA might find instances where we did not do what we were supposed to do, and we might get cited for

"I am excited to see what the new AI app will look like in 6–12 months. We have only been working on it for a few weeks and a prototype can already answer questions based on documents uploaded into the system."

this. In the worst case, a program might even get suspended. To sum up, there are costs and risks associated with the inefficiencies of not having an easily accessible knowledge base. If we had a customized AI-powered chatbot, we could, for example, ask about the expiration date of a bone marrow product, and it would provide an authoritative answer based on the relevant documents. For now, AI platforms like ChatGPT would probably give a correct answer based on federal regulations, but we would not be able to guarantee their certainty and it wouldn't know anything about our internal workflows.

One of the things that my group is working on is building an AI-powered chat application where one could upload documents that could be validated internally. This would ensure that the AI platform could understand the SOP or any other specific document, and answer questions correctly and accurately. It is a high-risk environment that we must tread carefully in, but once we get it right, it will be a powerful tool to help us make knowledge and compliance work less tedious. Ultimately, AI-based tools will help us spend fewer hours auditing our documents and give us more time to innovate therapies, which will consequently bring the costs of CGTs down.

Q Where else are AI and machine learning being applied to overcome challenges in supply chain management?

SK: Apart from AI helping us with knowledge and compliance work, it could also be used to manage contracts, which is one of the key activities in supply chain management. For example, AI tools could be used to draft quality agreements with specific vendors based on institutional templates. It could also verify what must be covered in a quality agreement based on each vendor's services, which could be very useful and save time.

AI could also speed up legal processes in supply chain management. Legal contracts often take a lot of time to complete, therefore having AI tools to carry out tedious rote tasks could make our lives easier and speed everything up.

Q How can digital systems enable the delivery of timely data to clinicians and researchers to accelerate CGT clinical research?

SK: Cellular therapies are living drugs that must oftentimes undergo cultivation in a growth medium. Timely data on culture quality and growth speed could help us automatically adjust the system, like adding glucose or washing out toxic metabolites.

However, building a customized digital system that enables the delivery of timely data in a clinical setting for CGT manufacturing can be complex and expensive. Therefore, I am looking forward to the next generation of semi-automated cell processors with built-in sensors

to continuously monitor the culture. Imagine you could track the temperature, pH, glucose, ammonia, and lactate levels in a culture automatically and continuously, which can vary from patient to patient. This will help us troubleshoot things when cells are not growing, and optimize the manufacturing process, thereby making it less costly.

Q What are your key goals in your own work over the next 12–24 months?

SK: First, I am excited about bringing some new CGT products online and developing processes for different kinds of therapies. For example, even though semi-automated cell processors are still in the beginning stages, we will focus on utilizing them to automate CGT processes. Additionally, we also aim to develop new hematopoietic stem cell-based CGTs for inborn mutations, amongst other products.

Lastly, I am excited to see what the new AI app will look like in 6–12 months. We have only been working on it for a few weeks and a prototype can already answer questions based on documents uploaded into the system.

BIOGRAPHY

STEPHAN KADAUKE is an Associate Professor of Clinical Pathology and Laboratory Medicine at the University of Pennsylvania School of Medicine, Philadelphia, PA, USA, and serves as the Associate Director of the Cell Based Therapy Laboratory at the Children's Hospital of Philadelphia (CHOP). In his role, he oversees the clinical development of novel cell and gene therapies, with a focus on automation to streamline manufacturing processes and reducing treatment costs. Dr Kadauke's work also includes leveraging AI to simplify compliance and optimize manufacturing processes. His efforts are geared towards making advanced therapies more accessible and affordable, particularly in global initiatives like the collaboration with the National Cancer Institute of Brazil. With a strong background in clinical informatics and transfusion medicine, Dr Kadauke is dedicated to advancing the field of cell and gene therapy through technological integration and interdisciplinary collaboration.

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CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN DIGITIZATION

CHANNEL CONTENT

INTERVIEW

Driving digital innovation for supply chain orchestration in cell and gene therapy

Abi Pinchbeck, Editor, *Cell & Gene Therapy Insights*, speaks to **Christian Fuchs**, Head of Orchestration and Exceptions Management for Cell & Gene Therapy (CGT), Roche/Genentech, exploring innovative approaches surrounding the digital orchestration of CGT operations. They also discuss the challenges of achieving scalable CGT solutions and the potential of implementing AI and machine learning technologies in the field.

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Q First off, can you tell me about your background and how and why you moved into the CGT space?

CF: I started supply chain management in Germany and spent roughly 7 years in pharma SC and digital consulting, before joining Roche in 2019 in Switzerland as a digital transformation lead in pharma technical operations. When I joined Roche, I began supporting the CGT industrialization program that was established to build missing critical business capabilities.

When Roche decided to form a dedicated CGT business unit in technical operations, I joined the unit and remained working on CGT. I now head the Orchestration and Exception Management (O&EM) team at Roche/Genentech and I am based on the US west coast.

Q What are you working on right now?

CF: We started the industrialization program in early 2020 with the goal of identifying and filling CGT-related capability gaps in Roche/Genentech to be able to commercialize our diverse CGT portfolio.

Due to my background and experience, I focused on supply chain topics that need to be addressed to always be able to globally deliver the therapies in a fast and compliant way, at the right time, to the right treatment site, and to the right patient. Data and transactions play a key role here.

I began looking into supply chain 'right to operate' challenges, like the chain of identity and chain of custody, with the goal of not just achieving compliance, but also enhancing the required capabilities to orchestrate the supply chain. Particularly for supply chain orchestration, it is not sufficient to focus solely on the 'happy path', as exceptions are the standard in CGT, which is why standard operations must be able to cope with exceptions from the business side. The work led to the formation of two program work streams that were merged later into one and were branded as O&EM.

After assessing gaps for O&EM and additional conceptual work, my work with the team changed into driving the discussion with our executive sponsors and IT counterparts towards the investment in an orchestration system to fill the identified gaps. Market research and assessment of available internal capabilities made clear that although some existing elements could be leveraged, (co-) innovation was the most promising way forward. From that discussion and after a few internal rounds to secure the funding, we began an investment phase where we started to design and implement the software systems that we are now building in collaboration with SAP (SAP Cell and Gene Therapy Orchestration) and Accenture (Intient Unify Treatment Center Portal). Last year, we realized a minimum viable product (MVP) version of the orchestration platform that we are now leveraging for clinical therapies and a commercial gene therapy. The solutions are now available as living products for other pharma companies, too.

Now as a team, our focus is on evolving from being a project and capability-building focused team into a functional team with business process accountabilities. We are part of CGT end-to-end value chain and cover globally three core areas for clinical and commercial with three teams: transactional operational execution with a focus on ERP transactions—known as the virtual treatment journey; master data and labeling readiness to execute the transactions; and internal digital product ownership of the orchestration platform. What keeps me busy is leading the team and ensuring we have everything we need to run the virtual treatment journey and that we adjust and adopt alongside the growth and changing needs of the portfolio.

Q What are the key challenges facing the CGT clinical and commercial operations and supply chain management space?

CF: Some challenges are related to the scope we cover, some exist due to the nature of CGT, and others are linked to the business environment we are operating in.

Our scope covers clinical and commercial stages, which are two different worlds in the pharmaceutical industry. For some therapies, we focus on both stages at the same time, which is a challenge as there are differences in some of the requirements. Our main goal is to support the

"...currently, interfaces from manufacturers with couriers in most cases are designed and implemented case-by-case and point-to-point. A standardized interface definition with an open interface infrastructure would help to reduce implementation cost and speed."

seamless transition from the clinical to the commercial stage. Also, our scope goes beyond the classical tech ops scope as we are directly collaborating with treatment sites.

Regarding challenges related to the business environment and the nature of CGT, the whole organization is rapidly evolving, meaning that there is a lot of innovation that causes uncertainty. We are talking about therapies that are new for the company, that require new supply chain models, that we enable with new systems, that require new data, that we deliver and run with a new organization. We must cope with this uncertainty in all areas, which involves pivoting from one CGT therapeutic modality to another and managing changing timelines within this dynamic business environment. This is in contrast to our traditional biologics business where, in simplified terms, there is one manufacturing technology with one core supply chain model (make to stock), as for each CGT therapy type there are different manufacturing technologies involved and various supply chain models. This can make pivoting from one product to another challenging, especially when it involves IT investments, but I am proud to say that we successfully managed to pivot between fundamentally different modalities in a short time. This was only possible because we focused on modularity, configurability, and integrability right from the beginning following a platform approach.

On top of that, there are challenges specific to the CGT field. To name a few, there are new requirements for how the product is treated and shipped, slot scheduling challenges, regulatory label-related requirements, and reimbursement-related challenges all of which are brought by the new modalities.

Q How can virtual orchestration and digital value chains help to overcome these hurdles?

CF: It all starts with the people and the mindset. It is crucial to find a trustworthy team that can be successful in such a dynamic and innovative environment. Entrepreneurial people who can work in an uncertain setting and can successfully navigate topics in complex organizations are the key to overcoming these challenges. In my eyes, this in combination with the support and empowerment of our higher management is critical.

There must also be the right system support to ensure compliance and to scale, which is why we invested in an orchestration system that allows us to run the portfolio. It is clear that you need to reach a certain level of process maturity to be able to design the system and find that sweet spot between too early and too late is essential. Otherwise, one might end up with functionality that does not enable the business process or a manual process that does not scale and consumes resources.

As mentioned previously, it is important to build an orchestration system with certain platform capabilities, such as modularity and configurability, as it is uncertain how the portfolio

will look in a few years. IT investments typically come with a certain lead time that necessitates sufficient configurability and flexibility. That ensures when a change in the portfolio happens, it is also reflected in the system within a very short lead time. The industry is still very young and the degree of standardization for orchestration is low. This also drives complexity and uncertainty.

People with the right mindset and flexible system support are two key things that helped us adapt to portfolio changes in the past, and are now helping us move toward successful commercialization.

Q Can you tell me more about your work in building and operating digital value chains for CGT?

CF: The biggest aspect of my work in the past few years was managing the IT investment into our orchestration system from a business side with the Product Owners, which aims to bring the right capability to the CGT environment.

A key difference from previous market solutions is our belief in multi-tenancy for our treatment portal as well as the fact that we did not build it in-house. Multi-tenancy means that the health care providers could use the front end regardless of the CGT product manufacturer. This approach would make it easier for health care providers to collaborate with manufacturers, as it would come with a certain level of process standardization and aligned data definitions, so that they only need one login to have a single overview of all their orders. They would work with one portal instead of having separate ones for each manufacturer, which currently drives a lot of complexity in some countries. We established this vision early on in the program, and we are engaging with industry partners to share, discuss, and gain support for our outlook.

Our goal is to develop the right solution both internally and for the market for the industry to grow. Together with Accenture and the Standards Coordinating Body, we kicked off a consortium for multi-tenancy, and have various engagements with other pharma companies that also have a similar vision. I am optimistic that moving forward, there will be progress in this area driven by customer need.

There are other areas in which a certain level of standardization would help the industry to grow, reduce costs, and increase efficiency. For example, currently, interfaces from manufacturers with couriers in most cases are designed and implemented case-by-case and point-to-point. A standardized interface definition with an open interface infrastructure would help to reduce implementation cost and speed. Also, treatment site and manufacturer collaboration processes could be standardized to a certain level to reduce room for error, training efforts, and complexity on the treatment site.

Q Where do you see novel AI and machine learning (ML) applications fitting into the picture of CGT orchestration?

CF: In general, there are various areas where ML technologies could be leveraged. At the moment, ML is more common in the scientific field, such as for analyzing pictures or researching large data sets. However, for us, we are in a phase where we are preparing for the future, e.g. by making the data accessible, however, we are still missing the necessary data volume in

operations to think about training ML models. Software such as intelligent chatbots could help with customer service and treatment site collaboration. Currently, the focus for us is on further enhancing the core solution with functionality to evolve the MVP. I am sure that this will be an important topic in the future. Nevertheless, we are trying to apply new technologies like automated testing and robotic process automation where we can to reduce manual efforts.

Q What are your key goals in your work and for Roche/Genentech as a whole over the next 12–24 months?

CF: Our main goal as an organization and a team is to bring our CGT therapeutics to the patients who need them. Bringing therapies to patients is the main motivation, and everything else links to it.

To bring the therapies to patients, we want to have successful trials and commercial launches, but how we do so within the organization is important. It is crucial to do so in a way that also works well for the team so that everyone is motivated and excited to work in such an innovative environment. We are further growing with the portfolio and for me, it is important to sustain our team culture and core values. It is critical that the growth is healthy, which is why we are exploring various models that allow us to keep flexibility.

Regarding the orchestration platform, we are continuing our path by expanding the MVP and scaling it into new regions, using it for more therapies, and enhancing it with additional functionality. I am excited to see that the software products that we co-innovated with the software provider gained traction in the market and I am optimistic that this will lead to collaboration opportunities within the industry as a user community, and eventually might help drive standardization.

BIOGRAPHY

CHRISTIAN FUCHS is a Director at Genentech, South San Francisco, CA, USA heading the global Orchestration and Exceptions Management team in Pharma Technical Operations Cell & Gene Therapy. His work is focused on capability building and operational execution, leading the teams that are accountable in clinical and commercial supply chain for transactional execution, data readiness, and the orchestration platform. He has a background in consulting and received his MSc in Supply Chain Management from University of Cologne, Cologne, Germany.

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CELL & GENE THERAPY INSIGHTS

LATEST ARTICLES

CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

iPSC-ing into the future of cell therapy: expansion in a hollow-fiber bioreactor

Molly Tregidgo and Nathan Frank

Induced pluripotent stem cell (iPSC)-derived therapies offer unique opportunities in the pharmaceutical industry and clinical practice to shift cell therapy into an allogeneic paradigm. The development of these therapies requires high-quality iPSC banks generated via standardized workflows to minimize starting material bank-to-bank variability. In this article, two expert scientists share their experience working with iPSCs, the associated challenges, and the workflow and protocol optimizations needed to achieve doses relevant for clinical studies or further manufacturing. The transition from manual to automated iPSC expansion is described, followed by an exploration of how to effectively expand high-quality iPSCs in a functionally closed, automated, and scalable system using hollow-fiber perfusion technology.

Cell & Gene Therapy Insights 2024; 10(7), 327–338

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FROM MANUAL TO AUTOMATED iPSC BIOPROCESSING

iPSC bioprocessing poses several challenges, not the least of which is that iPSCs are highly sensitive to changes in the microenvironment, meaning uncontrolled or poorly controlled settings will lead to differentiation into inappropriate cell types. During production and expansion, tight control of critical process parameters is required. In addition, high yields of cells are required for treatment with allogeneic iPSC-derived cell therapies, which often target large indications. The high yield requirement is limited by the fact that iPSCs are typically cultured in T-flasks, which have low throughput and require manual processing. Increasing the number of T-flasks

increases the risk of operator-induced process variability and contamination. Moving away from T-flask expansion is necessary to enable the generation of high-quality iPSCs for cell banking applications or as starting material for further expansion and differentiation.

One way of moving away from manual processing is to automate 2D expansion, utilizing the Quantum Flex™ Cell Expansion System. This system can be used to modify and expand the selected cell product to therapeutically relevant doses. Quantum Flex is a hollow-fiber-based bioreactor system that utilizes an incubator chamber and a series of pumps to expand cell populations in the lumen of the hollow fibers at a range of scales. Terumo Blood and Cell Technologies has three offerings for hollow-fiber bioreactors (small, research use only [RUO], and standard) to scale from process development to commercial manufacturing. Applications of the Quantum Flex system include cell banking and implementation as an automated seed train for 3D expansion.

An example of iPSC expansion in Quantum Flex using the RUO bioreactor kit is shown in Figure 1. Over a 6-day expansion period, over 2 billion iPSCs were generated with a harvest density of approximately $2 \times 10^5/cm^2$. These iPSCs maintained high-quality improved potencies throughout the expansion, demonstrated by a flow cytometry panel, which included surface and intracellular markers. Considering a cell dose requirement of over 10^{12} cells/year at a harvest density of $2 \times 10^5/cm^2$, 300 batches in the Quantum Flex bioreactor would be sufficient to supply annual requirements.

Once methodologies were developed for expansion in the Quantum Flex system, they were applied to reduce the seed train burden for other platforms. This includes automating a 3D iPSC expansion platform (Figure 2). The Quantum Flex system was implemented utilizing a direct thaw method, completely omitting the requirement for expansion in a T-flask. The expansion achieved in the 3D platform was comparable regardless of whether expansion occurred in a T-flask or Quantum Flex bioreactor. Quality was maintained with high expression of pluripotency markers at harvest. The expansion in the Quantum Flex system was found to be a suitable automated alternative to seed train that generates high-quality iPSCs, facilitating further scale-up in 3D.

QUANTUM FLEX: SCALE-DOWN FROM RUO TO SMALL BIOREACTOR

The Quantum Flex system early adopter program allows access to the new Quantum Flex features, which include run reporting and the ability to write protocols to facilitate better manufacturing settings. In addition, the launch of the smaller bioreactor facilitates process development with reduced media consumption and consumable costs. The small bioreactor provides all the benefits of automated production on a smaller scale and is a good option for small-scale bank generation.

The process from the RUO Quantum Flex bioreactor was scaled down 10× into the small Quantum Flex bioreactor, using direct scale-down of process parameters including seeding density, coating concentration, and feed rate. Scale-down between the two bioreactors was successful and generated comparable doubling time and yield per cm^2 across several runs, maintaining high harvest viability.

A key difference between Quantum Flex versus T-flask expansion is that the monitoring of a T-flask expansion is typically achieved by daily imaging, whereas in the Quantum Flex system, the cells are grown within opaque fibers, so traditional methods of cell growth monitoring are not viable. Therefore, cell expansion is monitored via consumption or production of key metabolites. Determining the yield from metabolite measurements requires an understanding of cell line-specific factors, such as production and consumption rate, and a mass balance based on the rate of media perfusion into the bioreactor. Once

FIGURE 1

iPSC expansion in Quantum® Flex RUO bioreactor.

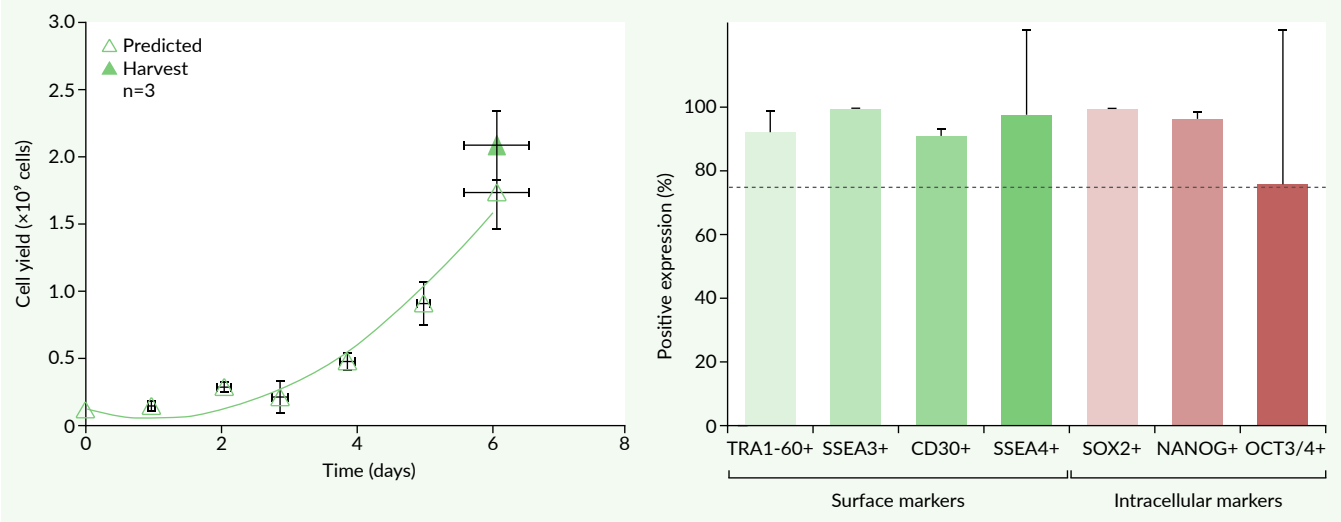
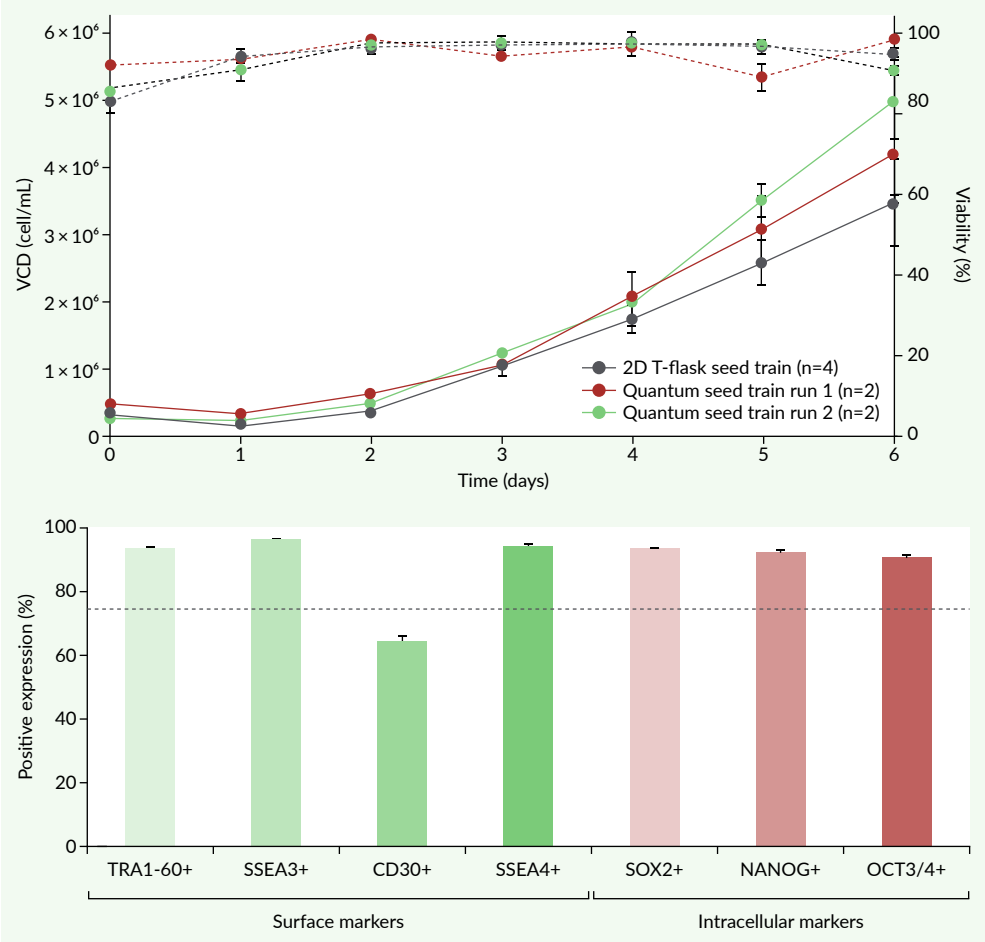


FIGURE 2

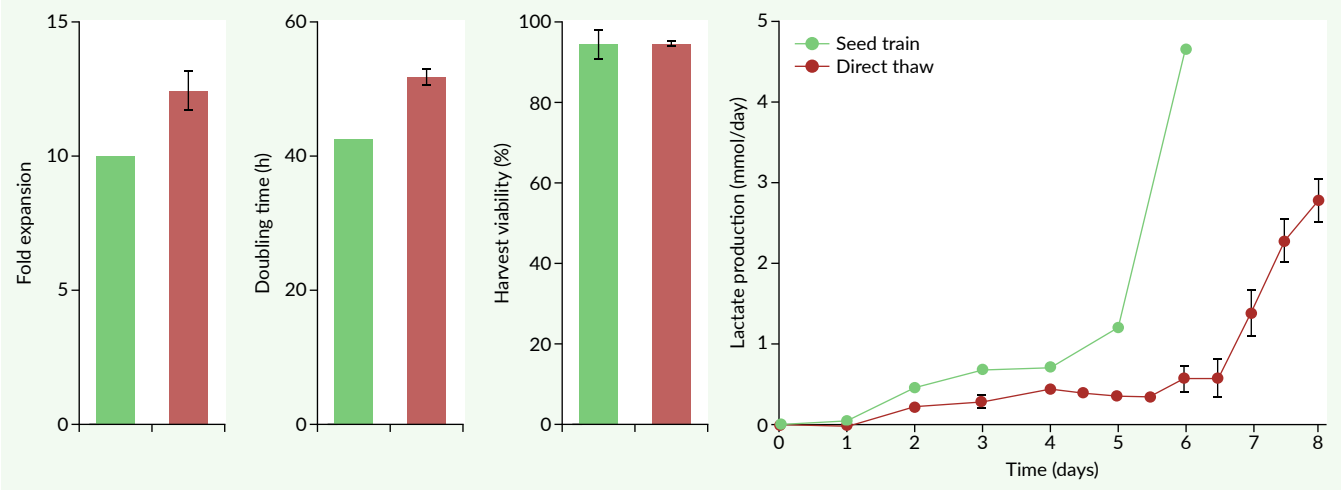
Quantum Flex RUO bioreactor as automated seed train for 3D iPSC expansion platform.



Dotted lines: Variability; solid lines: VCD; VCD: Viable cell density.

FIGURE 3

Direct thaw into Quantum Flex small bioreactor to close the seed train.



these models have been developed, they can be used to accurately predict the cell harvest density with a variation of ±10%.

Figure 3 illustrates the expansion of iPSCs in the Quantum Flex small bioreactor, with the n-1 expansion achieved using a traditional seed train process, or by utilizing a high-density cell bank, which was thawed directly into the Quantum Flex system. An increase in doubling time is observed in cultures that have a direct thaw because of an elongated lag phase in lactate production, which is closely linked to the expansion of cells. This is expected to be caused by the recovery after cryopreservation in direct thaw cultures. To ensure a high yield in these direct thaw cultures, the Quantum Flex harvest was postponed until a peak in lactate production was observed after 8 days of expansion.

Basing this harvest on a critical process parameter such as lactate production, rather than an arbitrary harvest day, resulted in an improved expansion in direct thaw cultures with no impact on harvest viability or pluripotency. An additional advantage of the direct thaw process was a reduction in overall culture time. Despite a 2-day increase in expansion, the requirement of a 2-week seed train was removed, resulting in a process duration of approximately half for the direct thaw cultures compared to the traditional seed train process.

MANUAL TO AUTOMATED iPSC BIOPROCESSING SUMMARY

iPSC expansion in Quantum Flex hollow-fiber bioreactors has been shown to provide an alternative to the T-flask for cell expansion, consistently generating high yields with high viability and pluripotency, by reducing the number of manual steps and reducing the requirement for highly skilled operators. In addition, direct thaw methodologies have been developed that demonstrate a reduction in the number of manual steps. While the implementation of these methodologies requires process optimization compared to the traditional seed train processes, the expansion demonstrated was comparable between the culture systems and with high viability and pluripotency.

iPSC EXPANSION IN THE QUANTUM FLEX SMALL BIOREACTOR

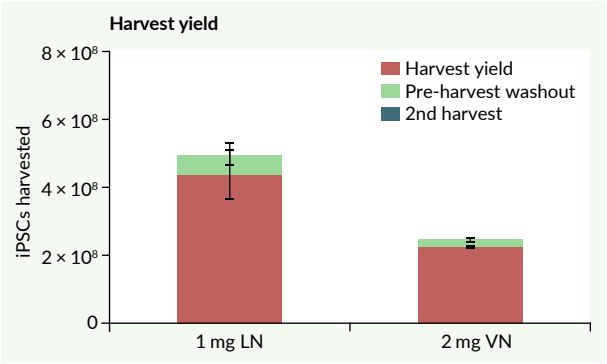
A loading procedure specifically designed for the Quantum Flex small bioreactor was developed involving a novel, automated method to

seed adherent cells evenly across the surface of the fiber membrane. In this method, the cell suspension was loaded into the bioreactor and moved back and forth four times while alternately reorienting the bioreactor from the right-side-up configuration to the upside-down configuration between cell spreading events to use the entire inner surface of the fiber lumen. This allowed multiple opportunities for the cells to anchor to the coated membrane surface with the intention of creating an even distribution of cells, so they had room to expand comfortably. Bidirectional flow was used to finish and move any unloaded cells into fibers. In a separate series of studies, this process resulted in a 75% increase in mesenchymal stem cell (MSC) yield relative to typical loading methodologies.

Prior to loading cells, the Quantum Flex system was coated with an adherence promoter to ensure that the cells could anchor to the membrane. For this, both recombinant laminin (LN) and recombinant vitronectin (VN) were explored. For practical purposes, a 3-day coating process was used to allow coating to begin on a Friday with cells loaded on the following Monday for a targeted Friday harvest; this allowed all procedures to occur comfortably during a typical work week. Coating was followed by a typical 4-day expansion period for iPSCs. On day 0, washout was completed with mTeSR™ Plus and CloneR™, and 15 million iPSCs were loaded into each system as single cells (7500/cm^2), using the novel loading procedure designed specifically for adherent cell culture in the small bioreactor system. Cells attached for 24 hours with bidirectional flow at ultralow flow rates (0.02 mL/min split 50/50 into each side of the fibers). Then, a gradual feed process was used to remove the medium and Rho-associated kinase inhibitor (ROCKi) passively without an active washout over the first 24 hours. A fresh bag of medium was added daily. Lactate readings were used as a proxy metric to determine cell expansion in order to adjust feed rates. At harvest, a gentle phosphate-buffered saline (PBS) washout was used. The washout fluid was collected for cell counts and characterization. 60 mL of Accutase™ was circulated for 4 minutes, and harvest from the small bioreactor was about 200 mL.

FIGURE 4

Harvest yield using LN and VN coating options for Quantum Flex bioreactor.



The results of this study are shown in Figure 4; 500 million iPSCs were expanded in the small bioreactor system using LN to coat, and 250 million iPSCs were expanded using VN to coat. LN yields were around two times higher than VN yields, though both options were shown to work. Some loss to the pre-harvest washout was noted—an average of 10–12%. Viability was greater than 95% on all harvests regardless of the coating material. A second harvest process was utilized to measure harvest efficiency, which was shown to be ≥98.4%.

When aligning metrics for manual 2D culture versus Quantum Flex culture, good alignment was seen between the LN-coated plates, the VN-coated plates, and the LN-coated bioreactor (Figure 5).

The lactate generation rate was used to determine cell expansion and subsequently increase feed rates as the expansion progressed. Lactate generation curves from the LN-coated systems were exponential, indicating good seeding distribution on that surface. A good correlation was seen between harvest yield and lactate generation rate on the day of harvest, indicating that lactate generation rates can be used to estimate the harvest yield of iPSCs in Quantum Flex.

FIGURE 5

Quantum Flex iPSC expansion kinetics compared with manual 2D culture.

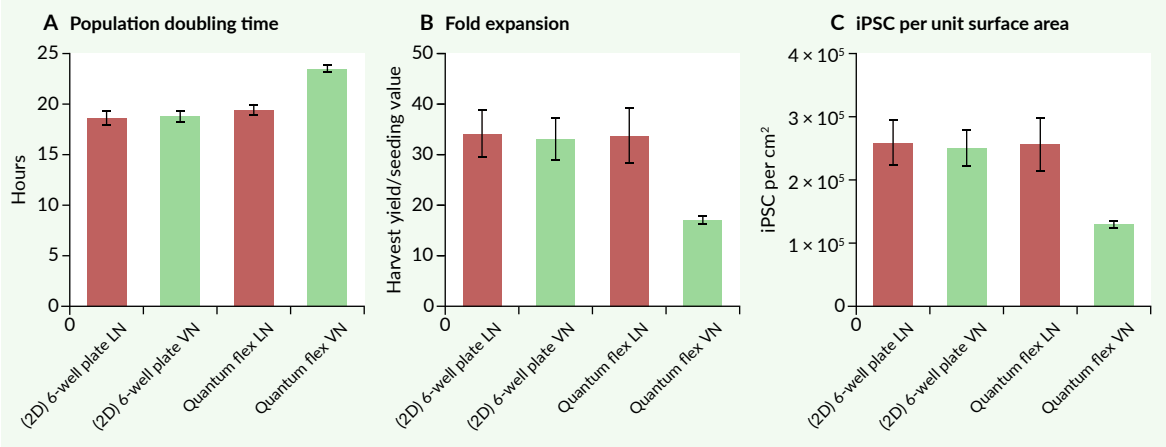
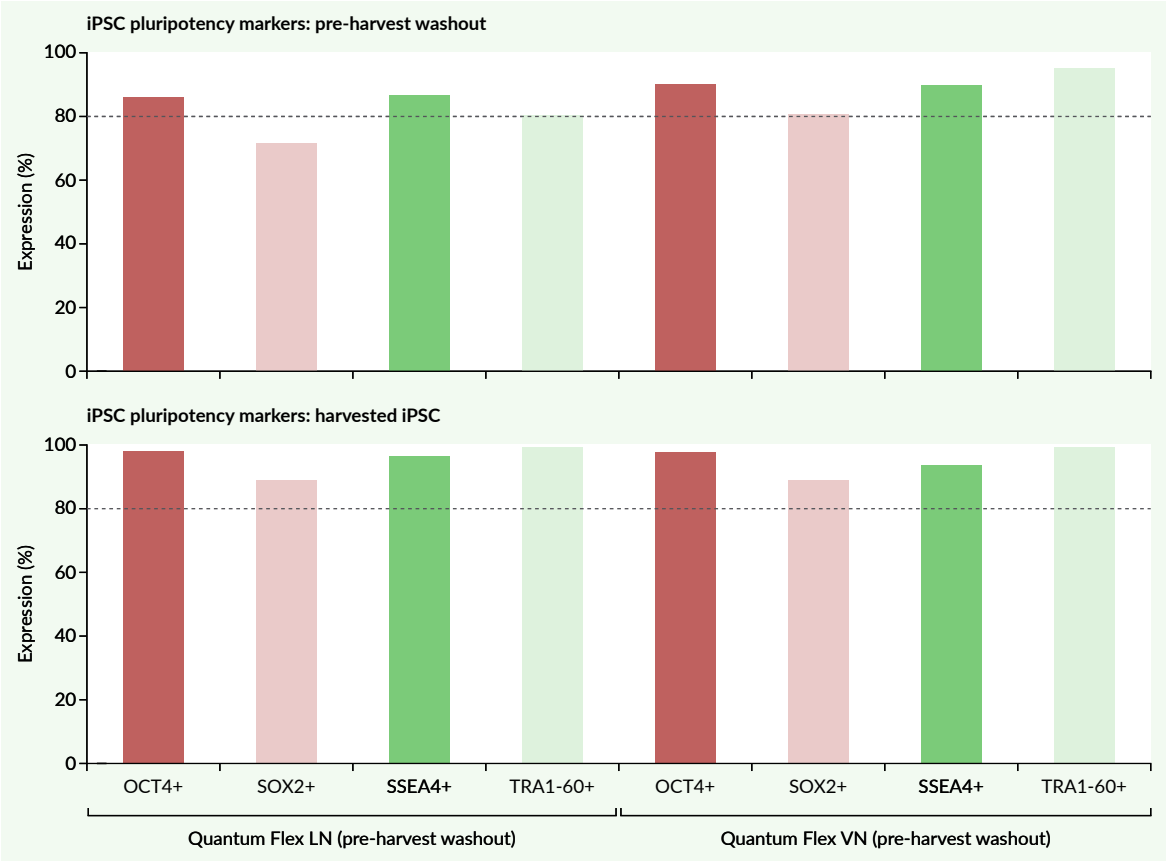


FIGURE 6

Assessment of iPSC pluripotency markers pre- and post-harvest.



To ensure the cells produced were of high quality, an assessment of pluripotency markers was performed. Figure 6 shows that pluripotency markers were expressed at high rates (over 90%) on iPSCs expanded in Quantum Flex, regardless of the coating material used. The iPSCs in the pre-harvest washout expressed markers at slightly lower rates, so pruning of these more differentiated cells may be advantageous in keeping harvest quality high.

SUMMARY

There is a large demand for automated, controlled iPSC expansion. The Quantum Flex Cell Expansion System can help fulfill this requirement as part of an iPSC manufacturing process to efficiently expand iPSCs. There are multiple coating options for the system, and iPSCs can be expanded to densities similar to those seen in manual culture. The iPSCs generated are high quality and maintain pluripotency markers at harvest and high harvest viability. Data has demonstrated potential applications for Quantum Flex; for example, in bank generation or for use as an automated seed train for further processing in 3D. This allows scale-up from smaller bioreactors to decrease costs and risk while maintaining cell product quality.

Q&A

Molly Tregidgo and Nathan Frank

Q Will the hollow-fiber perfusion technology also work for suspension cells such as T cells or CAR T cells?

NF: Yes. We have done extensive work culturing suspension cells, particularly T cells, in the hollow-fiber bioreactor. The counterflow containment method described, with flow going in equal measures on either side of the bioreactor, facilitates the movement of suspension cells in the hollow-fiber perfusion environment to enable adequate gas exchange and nutrient exchange.

This does not require coating the fibers. As there is counterflow containment going on, and the cells are inside the lumen of the fiber against the membrane that is transferring waste gases out, we can get very high densities of cells inside the 140 mL. We have had

30–50 billion T cells come out of the standard reactor. We do not want to be limited by the volume—these cells can be fed as much media as they require to keep thriving in that perfusion environment.

Q An increased lag phase is mentioned for the iPSC expansion of direct thaws in the Quantum Flex bioreactor. Is this the same as when culturing in a 2D flask?

MT: It is similar to what we see in a 2D T-flask. In T-flask culture, the first cycle has a longer growth phase compared to the following cycles as the cells recover from cryopreservation. This is slightly more amplified in the direct thaw cultures because, in addition to this, the cryopreservant is present. In our studies, the lag phase is highly similar across T-flasks and Quantum Flex.

In Quantum Flex, advantages include the larger volume. This means that if using high-density banks, cryopreservant can be diluted more to lower the impact. The perfusion system is also helpful for continuous flushing out of cryopreservant.

Q How do you monitor the confluency? Confluence may be critical regarding differentiation.

NF: We cannot see within the fiber as it is opaque, and so we do not monitor confluency the way someone would in a flask. Confluency monitoring is a subjective assay. Using lactate generation to indicate cell expansion is a more objective measure of cell confluency and cell expansion than putting one small portion of the flask underneath a microscope every day.

I have grown highly reliant on lactate values, specifically the shape of the curve. If lactate generation is exponential, that strongly indicates that our cell expansion is also exponential. After performing hundreds of adherent cell expansions in Quantum Flex, there is a moment where the curve goes from exponential to flattening. At that point, it is a strong indicator that you have hit confluency. Those lactate readings are very tightly correlated with confluency in the bioreactor, so an exponential curve of lactate generation can be relied upon to indicate the exponential growth of a healthy, expanding population in the system without looking at the cell confluency itself.

Q With the small bioreactor expansion protocol, what is the total amount of cell culture medium used over the 4 days? What is the highest cell density you have observed?

NF: The cells/cm^2 was 250,000 for LN and about half of that for VN, which correlates nicely with what we see in the 2D system. We used about 2.5 to 3 L of medium for these

expansions. We moderate media use based on what the cells are telling us they require. The runs with the LN were putting out more lactate, so I increased the feed rate closer to 3 L.

Q You mentioned that cell culture kinetics are monitored and highly correlated with the production or consumption of metabolites. How is this sampling done?

MT: Sampling is easy. Two types of sampling can be performed with Quantum Flex. There is an intracapillary side and an extracapillary side. Anything moving over the fibers into the extracapillary side can be sampled easily, and there is a one-way aseptic sampling valve to enable samples to be taken with a syringe. There is also a method to sample anything that will not be transported over, but the key nutrients for the kinetic modeling are predominantly found on the extracapillary side.

NF: As mentioned, you can sample from either the intracapillary or extracapillary side. For adherent cells, we typically sample from the extracapillary side because all the metabolites, glucose, and lactate easily pass through the membrane. Sampling from the extracapillary side should be representative of the entire system. For suspension cells, not only can you sample from the intracapillary side, but you can re-suspend your cells, take an amount of the intracapillary loop, and then count the cells/mL to track expansion going forward. Different methodologies and options are based on adherent or suspension cell culture.

Q Could the novel loading technique potentially create shear stress and be detrimental to the cells?

NF: This is something we do consider, and this is why we focus on reducing the rate of the washout to ensure we do not remove cells from the membrane. During the loading procedure, the movement of cells is in the tens of thousands of mL/minute/fiber. These cells are moving very slowly inside each fiber, so the shear stress is incredibly minimal. When we have measured shear stress even at higher rates, it has been negligible. The shear stress in the bioreactor is something to be aware of for certain niche cases, but it is not something that will damage cells most of the time.

Q In the future, will there be the possibility of scaling up the Quantum Flex for larger quantities?

NF: Although there have been no developments for this yet it would be exciting to have larger volumes in Quantum Flex. It is important to note that Quantum Flex already enables a high level of scalability through the use of small and standard bioreactors. The Cell Processing Application also allows the connection of up to 100 devices and enables protocol building and management across devices.

Q How does the Quantum Flex bioreactor take care of contact inhibition of iPSC mass culture?

NF: Contact inhibition goes back to cell distribution. We have strong indications that our cells are very well distributed inside the fiber based on various metrics. After testing countless populations of MSCs and iPSCs, we do not see any indications of over-confluency, such as flattening out of growth rates and a tendency toward spontaneous differentiation. We do not see any indicators of differentiation in the system.

Q Is it possible to induce differentiation of iPSCs in bioreactors (without any contamination), thus reducing the time lost in the lag phase for freshly thawed cells?

NF: That is certainly possible. I have heard of folks doing precisely that.

The incidence of contamination in our lab and among customers is incredibly low. With an adherent cell population, there are three open events for getting the adherent cell population from seed to harvest, and with suspension cells, there is one open event. There are very minimal opportunities for contamination to occur. The medium is all filtered before being put into the media bags, and the entire disposable set is functionally closed, meaning there are 0.22 µm filters on all entrances and exits. From personal experience, it is easy to avoid contamination with this system.

Q What are the advantages of having a 4-day expansion protocol over a longer protocol?

NF: I wanted to keep early process development close to what people are used to. The protocol was designed around making something that was practical and designed to be done in a work week. The process is designed to be automated over the weekend, to free up scientists from working over this time. It was designed to be feasible for customer use and made for people to have easy and low-energy use of the device.

Q Were either ROCKi or CloneR kept in the full medium throughout the expansion process? There is concern in the field about persistent exposure to ROCKi impacting iPSC quality.

NF: I appreciate that concern. Neither ROCKi nor CloneR are kept in the full medium throughout the expansion process. The initial washout before seeding the cells is done with a medium that contains ROCKi—in this case, CloneR at the appropriate concentration. Immediately after that seeding procedure occurs, the bidirectional attachment phase initiates, and the cells are moved to the membrane. The media provided at that point does not contain ROCKi, so the media is gradually replaced.

Q Is the small-scale Quantum Flex a straightforward scale-down model? The media composition might change over the length of the module depending on the scale.

MT: We originally used the larger bioreactors, and then swapped over to the small bioreactor. This is not a direct scale-down because there are similarities in the tubing sizes, for example. The processes were easily transferred and worked very well.

NF: These have the same membrane technology. The fibers in the RUO are differently sterilized which may make some difference in the fibers. The standard and small bioreactors, however, are completely identical. The only difference is the length and the number of the fibers. The extrusion is identical with the same 200 µm lumen and the same porosity. There may be some differences based on length, but we have not seen huge differences in the resulting cells if cultured using similar methodologies.

Q Are the hollow fibers single-use?

NF: Yes. The hollow fibers are single-use disposable. The system was designed this way to ensure the utmost good manufacturing practices (GMP) compliance for large-scale production.

Q Sometimes during a differentiation process, various media compositions are required. Is it possible to perform a full medium exchange to replace compositions?

MT: Yes, it is easy to change your media. You can fill your bag with whatever media you want, and then attach it onto your system with a welder. There are many different pre-programmed options to do a full media exchange or slow media exchange. Whether you want the media changed instantly or over a certain number of hours, it is possible.

NF: You can do a very gradual adaptation of cells from media A to media B if necessary. You can also consider the sheer forces and do it at a high or a low rate depending on what works best for your cells.

BIOGRAPHY

MOLLY TREGIDGO obtained her degree in Biochemical Engineering from University College London in 2015, where she later completed her EngD investigating novel scale-down models for perfusion bioprocess development. She joined Cell and Gene Therapy (CGT) Catapult in 2021, as a Bioprocessing Scientist. At CGT Catapult she works in upstream bioprocessing with allogeneic cell therapies across a range of bioreactor platforms, specializing in scale-up/down of cell culture processes.

NATHAN FRANK has more than a decade of experience optimizing parameters for cell and gene therapy production and scale-up. He has specialized expertise in hollow-fiber

bioreactor process development for a range of cell types aimed at various applications in regenerative medicine and immunotherapy. He is a two-time National Science Foundation Fellow and has a passion for teaching and communicating science to a wide range of audiences from established professionals to middle and high school students who are just beginning their scientific journey.

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CELL & GENE THERAPY INSIGHTS

INTERVIEW

Preparing for tomorrow's cell and gene therapies today: lessons from 30 years in the field

For the first time in history, cell and gene therapy products are reaching the market in waves rather than in one's and two's, creating unprecedented challenges for pharma/biotech and healthcare sector stakeholders alike. **David McCall**, Senior Editor, BioInsights, speaks to **Eric Faulkner**, President and Chief Strategist, Passage Health Associates, about the chequered history of market access in the advanced therapies field, and what lessons can be taken forward to ensure a successful and sustainable commercial future for the sector.

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Q Having worked in advanced therapies for nearly three decades and across hundreds of strategic asset scenarios, what do you see as the greatest challenges in the industry then versus now?

EF: The first approved gene therapies faced much the same challenges as the first cell therapy products that preceded them. These early advanced therapies were introduced into reimbursement systems that were never designed for transformative, single-administration therapies. Every aspect of therapy development, supply chain, evidence development,

reimbursement, and provider-side dynamics had to be considered. Many of these challenges still exist, similarly to the dynamics of drug-diagnostic combinations in precision medicine.

As the pioneering transformative gene therapeutics began to emerge, the considerations for commercialization increased dramatically. Preparing to launch these products required twice as much work, because companies had to figure out how to address the absence of an established reimbursement model, and determine what evidence needed to be developed as pricing for some of these therapies became more tangible. Because all of the manufacturer 'value capture' centered on a single administration, this was an entirely new business model for biopharma, presenting its own challenges of profitability in non-recurrent treatment scenarios. Additionally, the early advanced therapies had higher educational requirements for payers, physicians, and patients alike when they first started to emerge compared to the present day.

In the early days of the field's development, the US payers would categorically refuse to cover the costs of gene-modified cells, for example, because these approaches were so new and poorly understood. The thought of altering genes and putting them [back] into people seemed like science fiction. Although that hurdle has now been overcome, back then, there was a lot of uncertainty around how the market would react to something so novel. Pricing in this area was also a huge shock for all stakeholders and remains so today. We have all seen prices increase since those early days. For example, I have been affiliated with a US payer-provider executive organization working on CGTs. They were astonished by how quickly the prices of certain therapies went from US$500,000–US$2 million, then to US$3.5 million, and now bordering US$5 million for some therapies.

The broader community is growing more accustomed to thinking about valuing single-administration therapies compared to the traditional chronic disease-based models involving repeated dosing. However, 25+ years later, stakeholders still tend to have a hard time getting past 'sticker shock' and even when shown cost and impact data over time, are challenged to think beyond the conventional paradigm where expenditures are spread out over time. There is still more educational work to do there.

From a health economics standpoint, CGT costs can often balance out over time compared to some chronic treatment regimens, particularly involving biologicals or enzyme replacement therapies. However, we are still on a learning curve here and as we migrate into more chronic conditions, the value demonstration challenge will become more complex. Products have already failed or stalled in the market because sufficient focus was not taken to link surrogate outcomes to 'hard', longer term outcomes like severe morbidity, hospitalization, and death.

Another key challenge relates to inadequate investment in value demonstration. The main drivers of value are magnitude and duration of effect. This means that while traditional clinical trials can sometimes capture magnitude and transformative effect, they cannot show duration. Plus, some advanced therapies can promote health outcomes that keep improving well after the traditional clinical trial window. Even with 20 or 30 novel gene therapies coming to the market in the next couple of years, companies in this space still do not invest sufficiently in value demonstration. They often neglect to invest in real-world evidence (RWE) and do not treat the product as if it is going to launch immediately after Phase 2, especially if it is on a regulatory pathway for fast-track approval. Once a therapy receives market approval, especially

"In my experience, clinical trial evidence is only about 25% of the value proposition for CGTs, with RWE and long-term follow-up constituting the remainder of the value proposition."

conditional approval, there is a pressing need to then demonstrate its long-term durability and cost-efficiency, and companies are often not ready to address this necessity. In my experience, clinical trial evidence is only about 25% of the value proposition for CGTs, with RWE and long-term follow-up constituting the remainder of the value proposition.

Many companies also do not tend to invest sufficiently and early enough in natural history to characterize unmet need, build the field, and truly understand what transformative effect means for a particular scenario. All this investment has the potential to go right to the bottom line for a new therapy by better characterizing value, particularly when treatment alternatives exist. We also are operating in an era where emerging data sharing, network building, and AI can improve and integrate evidence development processes that were 'formerly sold separately'.

Trial designs and appropriate study methods have also represented a challenge here. Regulator, HTA, and payer organizations can be slow to move beyond historical evidence assessment approaches. While randomized controlled trials (RCT) are often still needed in many cases, in some areas like niche oncology and rare disease indications, single-arm studies make a lot of sense, but have had differential acceptance. When the population is small and has severe or fatal outcomes, it can be surprising when agencies still ask for an RCT given some of the patient realities, ethical considerations, and ability to leverage technology/methods to support sufficient indirect comparison. New avenues are also possible, such as basket studies (e.g., in sufficiently analogous diseases where a gene and/or cell therapy platform is established as safe, and the core difference may be the gene insert).

Staying on the theme of value demonstration, another critical issue from the very beginning of the CGT field was that much of the focus and dialogue centered around the concept of curative treatments. The word 'curative' brought its own set of challenges in terms of setting expectations with payers and other stakeholders instead of focusing on the transformative effect of advanced therapies. This scenario tainted payer expectations in scenarios where the therapy had a profound disease 'knock down' effect but might not be curative, creating the potential for combination treatment scenarios and for re-treatment. As a geneticist, I know that even with the so-called monogenic therapies, there is often much more going on under the surface genetically than is often acknowledged. There are many challenges, including variability, subpopulations, and other complexities. Part of addressing these complexities is for all stakeholders to understand that a curative effect may not always be achieved, or may only be achieved for a small portion of the population.

Lastly, an important current commercial challenge, particularly in niche treatment areas such as ultra-rare diseases, is that companies may not have anticipated the possibility of treating all or nearly all of a prevalent population. This concept turns both the financial and commercial models on their heads and can make a challenging business case for large and public sponsors

that are measured by driving shareholder value. In some many cases this has resulted in some of the vanguard large pharma companies taking a step back from how they were originally investing in gene therapy. Some failures in achieving market access—in Europe particularly—have been a major concern for many in the CGT space. For a time, Europe was like the elephant graveyard of advanced therapies, though we are beginning to see some breakthroughs now that therapies are coming in force.

There have been some substantial successes. Zolgensma® has been a strong global success case, as have CAR-T therapies, even though some would argue that the later were underpriced and hit bumps coming to market because sponsors did not fully understand the value proposition for inpatient therapies where costs are more bundled. On the other hand, the recent study by Mark Trusheim from NEWDIGS showed that from a regulatory standpoint, these niche, single-administration therapies are two to three times more likely to receive regulatory approval than other, more traditional types of therapies. The community is still working the business models out.

Q And what are some of the key opportunities, both then and now?

EF: One aspect that has remained the same throughout recent decades is the potential of these therapies to truly change people's lives. We have seen plenty of breakthrough advanced therapies coming through that can for the first time address the root causes of many diseases.

Such transformative CGT products raise the bar for all therapeutics, particularly those targeting aggressive acute or chronic conditions. This will push other companies, such as those working with small molecules or other biologics, to 'up their game' in order to achieve success. In essence, CGT products set a new standard for what constitutes a 'good' therapeutic intervention, and as a result, other more traditional therapeutics will need to adapt or risk falling into a very different usage environment than was the case in the past.

We are now seeing some significant changes happening in CGT manufacture, such as the emergence of decentralized or distributed manufacturing models. At the recent ISCT meeting in Paris, there were presentations and exhibits of at least a dozen different decentralized manufacturing systems in development. When these systems finally become mainstream, they have the potential to disrupt the industry in unprecedented ways. For example, these decentralized manufacturing systems may change the model for rare disease treatment – an area where we have seen some big pharma companies pull back despite increasingly transformative therapies reaching the market. The COVID-19 pandemic also had a major impact in that the industry is much more risk-averse now, reverting more proportionally to broader, conventional indications where the business models are clear, rather than focusing on rare disease and niche indications. The advent of mainstream decentralized CGT manufacturing may offer alternative avenues to help these patients.

Furthermore, the strong growth of the sector has pushed some aspects of the healthcare system to catch up, evolve, or be reimagined altogether, including in terms of developing viable reimbursement, payment, and evidence models. For example, the US FDA and the EMA are increasingly incorporating RWE when evaluating products, and some payers are now considering it more than ever before, too. A recent FDA guidance, for example, recognized decentralized evidence networks (that will use federation and AI) as a way forward to overcome and address value demonstration issues that were previously challenging or in some cases, impossible to address in a siloed dataset/system environment without incentives or processes to link and work across islands of data.

Ultimately, I believe these developments will create more opportunities for CGTs moving forward, making it easier for them to become more mainstream as the COGs decrease.

Q You had a unique experience being part of the team that helped take Zolgensma across 55+ markets and achieve double blockbuster status. What are some of the things that helped make that product launch so successful?

EF: The people who worked on the roll-out of Zolgensma had a real 'trial by fire' masterclass in launching a gene therapy product, and they have seen things that nobody else in the CGT industry has seen to date. The extended Zolgensma team, across timepoints, had to collectively navigate multiple first-in-world scenarios for broadly launching an advanced therapy. If you would have asked me a couple years before, I would have said what they achieved could not be done…but it was.

One of the key factors in our success was the organization's culture. For example, despite being acquired by large pharmaceutical companies, some biotechnology organizations manage to retain their entrepreneurship, and their ability to launch their own products and to grow. This was very much the case at Novartis Gene Therapies, formerly AveXis.

I think these acquired biotech companies can sometimes have the best of both worlds, culturally speaking—they remain an entrepreneurial group unburdened by bureaucracy that can stifle decision-making, but they benefit from the capabilities and muscle of a big pharma company to take these products across multiple markets around the world successfully. At Novartis Gene Therapies, there was a unique culture of focusing on collaborative problem-solving, irrespective of what internal team you happened to sit within, which was part of the magic behind the success.

Launching Zolgensma in over 55 markets in just 3.5 years required many completely unprecedented decisions to be made. Zolgensma is not only the most successful CGT product launch, but it was also one of the fastest, this is fascinating, especially considering its cost of around $2 million. I don't believe any of the other commercial CGT products, including the CAR-T cell therapies and products such as Luxturna®, saw as much ground covered in the same

sort of timeframe. In fact, Zolgensma was actually one of the fastest product launches of any therapeutic product in history.

It would be very interesting to know if such a successful launch could be replicated in a more traditional big pharma model. The commercial experience and muscle of the big pharma organization was key, but so was the entrepreneurship, flexibility, and willingness to take risks that the biotech element brought to the table. In general, though, Zolgensma did benefit from the fact that it was such an obviously effective therapeutic for a pediatric indication with severe unmet medical need. As the CGT field moves into more chronic indications, the complexity and scale of the commercialization challenge is only likely to increase.

Importantly, the developers of Zolgensma invested heavily in RWE, to the same level or even greater than the investment made in clinical development. Part of the team had deep foresight on what would likely be needed and convinced leadership to invest—which was critical to even being able to commercialize an advanced therapy in many markets. As a result, we ended up building the largest evidence engine for a rare disease in the world. This was absolutely necessary in order to meet all of our various data requirements: we needed to supply a 600-page dossier to five different regulatory bodies, we leveraged RWE recurrently to support payer and HTA asks, we used data to support risk-sharing agreement negotiations with payers, and leveraged evidence to needed to support value-based pricing across various markets. When you have a therapy that costs US$2 million, payers won't just take one look—they will scrutinize it multiple times and keep looking. Without investing in RWE, we would certainly not have been able to support the rapid scale-up and launch of Zolgensma across multiple markets—it was pivotal to our success.

As a specific public example, the Federal Joint Committee (G-BA) in Germany, who a year or two prior to this would not have even considered RWE, requested one of the most sentinel comparative studies in history looking at Zolgensma, Spinraza®, and Evrysdi®. Had Novartis not invested in the registry data and established partnerships with other registry holders, the organization would have lacked sufficient data to meet the G-BA's request, which would in turn have impacted Zolgensma's overall success. At the time that you find out that you need evidence and have not invested in the advanced therapy space, it is often then too late. This has been the case for every advanced therapy that has either failed or had major uptake problems or delays. However, even large pharma companies are rethinking how they invest in evidence in an integrated manner as new products are more frequently coming to market without sufficient evidence to avoid delays and restrictions.

Q As the number of CGT launches increases from a very few to many, how does this change the dynamic? What do companies need to focus on to succeed in a more competitive environment?

EF: The past 12 months have constituted a watershed year in terms of the sheer number of CGT product approvals. Previously, there were only a relative handful of such therapies

"Emerging markets that take on advanced therapies without the operational baggage of established markets with more circumscribed and almost immovable processes may be able to lead the way..."

approved each year, and payers did not pay them as much earnest attention given their transformative nature and limited overall budget impact compared to other therapeutic technology areas. However, now that CGT products are coming to the market in a consistent wave, we can expect the scrutiny and management practices to increase. People also talk about affordability concerns, but we have not really seen a comprehensive analysis that looks at how much we might save from advanced therapy treatments that reduce or forestall the need for recurrent, lifelong treatments.

We have also seen some interesting things bubble up. For example, one of the top five private health plans in the USA, Cigna, recently introduced a scheme whereby if you are unfortunate enough to have a serious disease and there is a CGT treatment available, your plan will only cover it if you specifically pay a fee for that coverage. This new approach represents a health plan's response to the increasing number of commercial advanced therapies by shifting some of the financial responsibility to the patient. There is also evolution in how CGTs are handled at the regulatory and payer levels that can both help advance and have potential to restrict these therapies. Changes are already happening to better manage these therapies, and will continue, but not yet in a sense where stakeholders step back and think about what single administration therapies really mean to health system operations versus what has come before. Emerging markets that take on advanced therapies without the operational baggage of established markets with more circumscribed and almost immovable processes may be able to lead the way there.

As we move into chronic disease areas, pricing for CGTs will become even more complex compared to rare disease therapeutics. For example, an advanced therapy addressing even a tiny fraction of the overall market for diabetes products would generate far more revenue than Zolgensma, even if it were priced anywhere within the brackets of CGT oncology products. However, to prove the safety and efficacy of CGT therapies for many chronic diseases, it will be crucial to ensure you understand the connection between a surrogate and a hard endpoint. That involves thorough preparation. You need to 'measure three times and cut once'. You need to think how you prove 'transformative effect' versus simply working from the outcomes script that you are given. You need to be nimble enough to retrench as necessary. Then, once you enter the competitive marketplace, it is important to understand how to build your market and develop your value proposition in innovative ways. We will soon see the first examples of having multiple commercial CGT products for the same disease—in hemophilia, for instance—and we will observe how that plays out. The key to success in such a scenario is to start early, integrate market access and other strategies from the very beginning of development, and think about comprehensive solutions, because operating in silos will lead to more product failures and setbacks for the sector.

Rethinking the investment model is also key to an integrated operational approach. If you skimp on your RWE investment and plan on only leveraging clinical evidence, you will have challenges and limited evidentiary tools to overcome them. Think of your RWE investment as your Phase 3 equivalent for all those fast-track therapies, with the exception that a registry or evidence engine is the publication gift that keeps on giving versus following a one study:one publication paradigm. We also have game changing technology platforms that are emerging that enable rethinking how one invests in evidence development in a more efficient, flexible, and high-yield manner.

Closely following the pricing and risk-sharing side will be warranted as the market becomes more crowded. The risk sharing agreements for the advanced therapies that have come to market to date aren't particularly fancy or unconventional because they haven't needed to be. 15 years ago, we were exploring various payment models, such as the reinsurance model and the so-called 'Netflix model' for amortized payment, that some researchers are rediscovering now. But while these models are interesting, they require changes in operating practices and even in law. Not easy things to change in the health system. Other approaches such as set-aside funds or cost-sharing models have potential, but we have discussed them for years and without a central organizing group such as the government or perhaps a global non-profit to champion them, may not see 'lift off' any time soon. Because of this, such approaches are for the time being highly risky, and will require a collective 'go-to' when and if the pain threshold becomes high enough as the advanced therapies market expands.

I believe that any payment strategy that fits, or is planning to fit, closely into an existing system is more likely to be successful, as evidenced by the initial vanguard of commercial advanced therapy products. It is important to keep a firm pulse on how advanced therapies and their pricing are being accepted. We are increasingly witnessing first-of—kind products being launched, where what has come before yield insights into what may come after. The key will be to learn from each new experience and use that knowledge to prepare for the future.

Q We see novel technologies like mRNA and gene editing products beginning to enter the marketplace. What do these mean for the CGT space?

EF: Oligonucleotide therapeutics and mRNA-based products, together with gene editing therapies, have the potential to significantly disrupt the CGT field. Generally, these products are cheaper to manufacture than more traditional advanced therapies, and they more closely align with the conventional recurrent dosing paradigm that pharmaceutical and biotechnology companies understand. Such therapies can also leverage more conventional discounting and other approaches to gain market share in ways that CGTs currently cannot due to COGs and single-administration value capture requirements.

These therapies also have the potential to multiplex, meaning they could target multiple genetic targets simultaneously—a critical step forward for a CGT world where we are current

addressing a shotgun problem with a rifle in terms of needing to hit multiple targets to address more complex diseases based on underlying biology. This could have a transformative effect on disease treatment approaches and therapy knock down effect. As a result, there may be increased pressure to integrate precision medicine strategies in order to identify which patients are more or less likely to respond to a particular therapeutic approach and potentially control costs where separately payable combinations may emerge.

Ultimately, I think we will see a marriage of precision medicine approaches in the CGT space as a cost-control mechanism, as overall budget impact of the sector expands. However, oligonucleotide and gene editing therapies have the potential to add transformative value whilst adhering to models that are more familiar to the industry, even where precision medicine approaches may also be leveraged.

Additionally—unlike CGT therapies, which permanently remain in the patient's body unless engineered with an 'off-switch'—nucleic acid-based therapies have a limited half-life. This means that if certain challenges arise, patients can simply stop the therapy and switch to alternative treatments.

Q If you had one recommendation to share with CGT developers that could help them make a new product commercially successful, what would it be?

EF: Planearly and prepare to retrench frequently...but do it together.

In the CGT world, you can't take anything for granted or get locked into siloed thinking (i.e., 'my role only covers regulatory', 'my role only covers clinical') if you want to optimize. For this space, success is the sum of the integrated parts and will be for the foreseeable future. Reliance on one endpoint or past evidence strategies will result in failure—it is a sum of the parts or a 'let us count the ways' model of value demonstration. You have to both think and act transformatively and in concert, while not losing lessons of the past. There are still many scenarios or decisions to come in CGT that no one has faced yet. Some solutions are novel, some are not. The field is learning, but the majority of stakeholders still have a narrow range of tools in their toolkit because deep experience in CGT is still rare. Leveraging the best parts of all and the collective knowledge base is key…as long as decisions are still made to move forward.

CGT developers must pull all elements of the development cascade (health economics and outcomes research (HEOR), RWE, market access, commercial) forward in the development cycle in an integrated manner to maximize success, especially regarding supporting pricing with value-based evidence. Being able to think upfront, identify how and for whom the therapy will be transformative, and how the product compares to other therapeutics on the market, is pivotal. In essence, developers must assess how much of a transformative effect will be required to get a single-administration advanced therapy to the market, especially if it has a very high cost and competes with other products. This pre-planning and thoughtful diligence is also mission critical in an environment of limited budgets and financial operating challenges, particularly in

the short-term where over 180 companies in the broader biopharma sector are laying off staff. Not planning investment for CGTs in an integrated manner is the present-day equivalent to chopping off your nose to spite your face…it may seem feasible in the heat of a moment, but it diminishes asset value and exposes one's flank to the full dangers of today's healthcare jungle.

As the field expands, it will become more, not less, challenging for CGTs to compete. Therefore, thorough planning, focusing on transformative effect in the short and longer terms, not taking anything for granted, considering all the options, and collaborating on the journey will all be part of the recipe for success.

BIOGRAPHY

ERIC FAULKNER is a globally recognized executive and business builder in innovative technology evidence development, value demonstration, access, and commercialization with over 30 years of experience. He is the founder of Passage Health Associates, a boutique partnership consultancy that creates bespoke strategy and solutions for complex health technology developers, payer and health systems, and governments. Recently, he has served as Vice President and Exec Team Member of Value and Access at Novartis Gene Therapies. Leadership roles have included value and access, HEOR, HTA, RWE, policy and end-to-end, integrated development, launch and commercialization services for biopharma, medical device and diagnostic companies at PPD/Evidera, IQVIA, RTI Health Solutions, and the Lewin Group. He has also advised US and EU governments, private equity investors, health non-profits, global think tanks, industry and medical associations on business, practical and policy implications of complex technologies. He serves as Assistant Professor for the Eshelman School of Pharmacy at the University of North Carolina at Chapel Hill and as Executive Director of the Genomics Biotech and Emerging Medical Technology Institute of the National Association of Managed Care Physicians (NAMCP).

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CELL & GENE THERAPY INSIGHTS

INTERVIEW

Navigating the European landscape of advanced therapy funding and commercialization

While Europe continues to fall behind North America in terms of the amount of funding available to advanced therapy innovators, opportunities still exist for biotech companies with a sound commercialization strategy. **David McCall**, Senior Editor, BioInsights, speaks with **Dmitry Kuzmin**, Managing Partner, 4BIO Capital, about the current financing, commercial, and regulatory landscapes for advanced therapies in Europe, and the implications for academia and industry alike.

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Q What are you working on right now?

DK: As the managing and founding partner at 4BIO Capital, I work on building and investing into Seed and Series A stage companies. We currently have a sizable portfolio that is progressing well, and a robust pipeline to continue investing in our Fund III.

Q Can you give us your high-level overview of the current status of the advanced therapies sector?

DK: It is a very exciting time because, as of last year, we now have an exit (e.g., a merger, an acquisition, or an initial public offering) and at least one US FDA-approved therapeutic in almost every modality of advanced therapy. This includes gene therapy, gene editing, various types of cell therapy, and various RNA medicines. We also have advancements in bi- and tri-specific antibodies, antibody-drug conjugates, T cell engagers, and even microbiome therapeutics. As a result, across the board, we now have success for investors, potential interest from big pharma, and most importantly, commercial rollouts involving actual patients, payers, and revenues.

Across the sector as a whole, depending on the evidence you consider, we currently have anywhere from 6–11 blockbuster drugs. Sales across the sector are also growing at approximately 25% per year. Furthermore, we have had some successful product launches lately, including Elevidys®—a gene therapy for Duchenne muscular Dystrophy—which appears to be taking off rapidly.

At the same time, we have faced some challenges, such as the recent hemophilia product launches. These challenges will guide future decisions, as they show us the diversity of the real-world evidence now being gathered. Overall, I have never been as excited about the advanced therapies space as I am today, as we continue to transform the sector into a crucial pillar of medicine.

Q How do you see the field evolving in Europe, specifically? What can we glean about its future prospects from recent investor sentiment and commercial and corporate trends in the region?

DK: An important thing to consider is the different attitudes taken approximately two decades ago by the UK MHRA and to an extent the EMA in Europe, compared to that of the US FDA. In the UK, we arguably had a regulatory head start, which in turn led to a research head start and then a corporate head start. For example, the MHRA's positive attitude towards this field led to many academic programs and investigator-sponsored trials, which then translated into the success of companies like Orchard Therapeutics, Freeline Therapeutics, Nightstar Therapeutics, and others. Europe has to a large extent capitalized on its lead in advanced therapies, as the region is faring much better than average compared to the broader pharma and biotech sectors. However, that head start is now gone. Today, the regulatory environment is very much a level playing field between the USA and Europe. As a result, the availability of capital—something in which the USA has the clear lead—has come to dominate and define this market.

"If you ask me what we can do to strengthen the European market in advanced therapies today, the answer is simply 'more capital'."

In Europe, we consistently see creation seed volumes at 40–60% of the US volume, which is very healthy compared to the historical 10–15% volumes. A healthy market is a rapidly progressing market, but now the relative lack of capital is coming back to haunt the European market. This is translating into lower biotech valuations and more productive opportunities for VCs like us, especially those building companies.

If you ask me what we can do to strengthen the European market in advanced therapies today, the answer is simply 'more capital'. We desperately need more capital across the board, more VCs in general, and more VCs paying close attention to this space. Additionally, we need more support from local partners across the board.

Q How are these trends reflected in 4BIO Capital's ongoing investment strategy and portfolio?

DK: Being a creator fund and an early-stage fund, we invest in the long-term climate, not the short-term weather. Market downturns and decreased capital availability do not bother us much because our cycles are relatively long by design, with an average holding period of 5–7 years. This allows us to account for significant cyclical movements during that time without rushing to market.

As a result, general market conditions do not influence our investment strategy. What really matters is real-world data feedback. As we go through successive funds, we test and verify hypotheses. Some are successful while others are not, and we learn from both our own and others' experiences. Today, our key considerations can be divided into business and technical aspects.

Starting with business considerations, we have focused more on Europe, particularly in the UK over the past eighteen months due to a convergence of strong science and a lack of capital in the region. We have done less in the USA over this period, although we recently closed an unannounced deal with a tier one fund in the USA and near to closing another. Currently, 75% of Fund III investments worldwide are in the UK, reflecting the lack of capital in Europe and the opportunity for integrative, holistic, and later-stage creation stories there. However, I expect this focus on the UK will decrease over time.

A significant business consideration, transitioning into a technical one, is the resistance in the community to the pricing of gene and cell therapies. On the other hand, we have seen the emergence of biologics and biological combinations in first-line oncology treatments. For example, first-line bladder cancer patients now receive a combination of a second-generation

"While there is still a place for commercialization of orphan therapeutics, it would be productive for the industry to push emerging advanced therapeutics into larger markets."

antibody-drug conjugate and a checkpoint inhibitor. This is revolutionary from a business perspective because these treatments have high cure rates and do not cost a lot of money, relatively speaking. This new landscape affects the commercial future for CAR-T cell therapy and also complex antibody-derived treatments like bispecific T cell engager therapy.

Because of this, we are considering what a win should look like in new preclinical development projects, particularly in oncology and hematology. Two key technical considerations guide us. Firstly, we focus relentlessly on CMC, particularly on reproducibility, product definition, and the cost of goods (COG). Over the next 10 years, anything that costs more than $20,000–$30,000 per patient to manufacture will not be viable. We therefore aim for single-digit thousand-dollar COG per patient for most of our therapeutics.

The second consideration is moving beyond orphan indications. While there is still a place for commercialization of orphan therapeutics, it would be productive for the industry to push emerging advanced therapeutics into larger markets. The main barriers to this are technical, and primarily CMC-related. Many current platforms are uneconomical for large-scale diseases. For instance, central nervous system (CNS) delivery of genetic medicines is not feasible with current technology, as only 1%–5% of the administered genetic material reaches the brain. This is not economical for diseases like Parkinson's or Alzheimer's.

Our focus is on making the manufacturing of these therapies reproducible, scalable, and automatable, as well as ensuring the products are easy to characterize. These goals drove our first publicly disclosed Fund III investment, ViaNautis, which we co-led with UCB Ventures and BGF, with participation from Eli Lilly and the Cystic Fibrosis Foundation. ViaNautis focuses on commercializing polymeric nanoparticles to deliver high proportions of systemically administered medicine to the brain. This innovation, from Prof Giuseppe Battaglia's lab at University College London, aims to drive advancements in CNS indications.

Overall, our strategy is informed by our desire to lower costs and make advanced therapeutics feasible for commercialization on a much larger scale than is currently possible.

Q It's been 4 years since you published a Commentary with BioInsights on **'Addressing the current limitations of AAV gene therapies'**—what is your take on the challenges and opportunities that particular area is being presented with today?

DK: As I previously mentioned, the main limitation right now is the high cost of goods. We need to work relentlessly to bring that down. Another limitation is tissue specificity. Most

current therapies target the liver, brain, and eye but we still need therapies for the heart, kidney, GI tract, and joints, for example. We are starting to see some increased efforts to target these areas, but it is largely being driven by innovation in the non-viral delivery space. AAV vector developers are feeling the pressure as even though non-viral methods do not yet enjoy the same degree of validation and success as viral methods, they are getting there.

Another limitation of AAV is in the gene editing space. AAV has been widely used for delivery of gene editing payloads, but it is perhaps not the ideal choice. To make the method more ideal, researchers are looking into engineering solutions to reduce AAV's cytotoxicity to hematopoietic stem cells. They are also innovating around AAV to secure different sources of donor strands for these approaches.

Q Both the regulatory and market access environments in Europe have come in for criticism from the advanced therapies industry recently—how do you see both pictures evolving, and what are some important related learnings for advanced therapy developers to take forward?

DK: To me, the main barriers in Europe are really about commercialization rather than regulatory approval. I think the EMA is fairly efficient for a large, federated regulator. The MHRA and FDA may be more efficient in some approaches, but generally, the EMA is on par. I do not see a significant problem in getting a well-characterized, well-trialed therapeutic through the EMA. In this field in general, the regulators are doing their jobs well. I dislike it when commercial people criticize regulators because their key mission is to keep patients safe and ensure only robust, well-vetted medicines reach the market. They do an excellent job, even if there is always room for improvement. Generally speaking, they are very good gatekeepers.

The real challenge is in the commercial rollout. In the UK, the National Institute for Health and Care Excellence (NICE) is often criticized, but they manage the cost of care in the NHS, which is a free-at-the-point-of-care system. It is important to remember that a $3 million gene therapy costs nothing, or almost nothing, to the UK patients who receive it. NICE has a strong mission to drive down costs, which is appropriate, and they have the added benefit of offering a single point of negotiation. By contrast, some genetic and cellular medicines in other individual European countries must satisfy multiple governmental agencies and obtain a number of different certifications varying in levels of relevance, which can be cumbersome.

Streamlining these processes would be amazing, but many of these are national issues rather than supranational ones. The EU has limited influence over national regulations despite decades of effort. Pharmaceutical regulation remains a national issue, with limited cross-border cooperation compared to, for instance, the intellectual property market. Major powers keep healthcare regulation and rollout as national issues due to their ties to tax, budget, and healthcare system organization. Healthcare will always be a political issue. Any reform will

encounter concerns about public cost and safety, especially from a genetic engineering perspective, which is historically significant in Europe. We need to be aware of these issues as our industry grows.

A long-term issue for the world as a whole, not just Europe, is the disparity in access to genetic medicines between developed and developing markets. Although there is a moratorium on germline editing, we are to all intents and purposes creating genetically different humans in developed markets. This could lead to social, religious, and political issues. A child born with hemophilia in Indonesia is significantly less likely to receive genetic medicine than a child in the UK. This creates a gross injustice and potential long-term instability.

Our industry in the developed markets needs to provide long-term thought leadership in working with developing markets. We must drive down the cost of goods, ensure proper commercial rollouts, and guarantee broader access to avoid social fallout and protect our industry's future globally.

Q Are there any other key pieces of advice that you can share for advanced therapy start-ups seeking funding in the current environment?

DK: It is a challenging funding environment because it is a market of haves and have-nots. The haves are raising large rounds, while the have-nots are not raising anything, leading to a more dislocated and bifurcated market. Therefore, pontificating on the do's and don'ts is not very helpful, but I will say this: data continues to be king. Having a robust, well-validated, and well-thought-through case addressing an unmet need will win in the long-term over pursuing a fashionable target.

I would much rather follow Julius Caesar's saying: be the first citizen of a small village than the second citizen of Rome. In this case, I would much rather be the first to market with a unique indication neither you nor I have ever heard of than the tenth to market with a gene therapy for hemophilia B. Objective evidence from the market shows that it is not a winner-takes-all situation, but the first and second products to market typically take a large chunk of the market, maybe with a third challenger. These are important considerations for startups when planning which targets to pursue.

Returning to the importance of data, having well-rounded, well-thought-through, and well-controlled experiments in a relatively complete package beats just having great ideas. This is a crucial point, especially for academic startup founders. Academics are the best at criticizing their own work, so taking a second-reviewer perspective on your paper, asking what else genuinely needs to be done, then doing that work in a thorough fashion is a recipe for success in a downturn.

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

DK: We have some important trial readouts coming up from our Fund II portfolio very soon. Over the next couple of years, we will see if our ophthalmic gene therapies, such as those developed by SparingVision and Ray Therapeutics, can truly halt or even reverse retinal degeneration and restore vision to the fully blind. This would be revolutionary. We are certainly looking forward to reviewing those data.

In addition, we are working on bringing gene therapy and gene editing to other organs. We are very keen on these developments and also on improving the targeting of both gene therapies and RNA medicines to specific cell or tissue types.

While we are enthusiastic about these specific technological advancements, our overall priority is still to deliver meaningful innovation to patients. That is our key goal.

BIOGRAPHY

DMITRY KUZMIN is managing and founding partner at 4BIO Capital, London, UK—a venture capital firm focused exclusively on the advanced therapies sector. At 4BIO, he leads the team of scientists, physicians, and former operators responsible for sourcing, evaluation, and post-investment management of both private and public investments. He is responsible for shaping and maintaining the proprietary technology strategy of the firm, the in-house research efforts and academic collaborations. Before Dima's time at 4BIO, he spent many years in academia, focusing successively on structural biology, neurochemistry, systems neuroscience, brain-computer interfaces, and gene therapy. He contributed to the foundation of the field of chemogenetics (control of excitable cells using a combination of gene therapy and small molecules). Away from 4BIO, he serves as an Assistant Professor of Medicine (Adjunct) at Yale University, New Haven, CT, USA. Dima is a Kauffman Fellow and a Fellow of the Royal Society of Medicine.

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CELL & GENE THERAPY INSIGHTS

INTERVIEW

Considerations to optimize and act upon long-term follow-up studies for cell and gene therapies




Elizabeth Donahue, Luis Arthur Pelloso, Dan Takefman, and Keith Wonnacott

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Q To set the scene, could you share your reflections on the evolution of long-term follow-up (LTFU) studies in the cell and gene therapy space to date?

DT: The administration of gene therapy products can result in long-term gene expression, potentially for the life of a patient. Prolonged expression of the transgene may be associated with long-term risks resulting from things such as malignant transformation or

> "There has been a downgrading of [plasmids, poxvirus, adenovirus, and AAV] products, but again, one of the primary drivers of LTFU is persistence." — Dan Takefman

autoimmune-like reactions. Additionally, some products have the ability to integrate into host cell genomes.

These are probably primarily based on gammaretroviral and lentiviral vectors, which have the potential to disrupt normal genes and activate proto-oncogenes, leading to malignancy formation years after product administration. Unfortunately, this is more than a theoretical risk, as insertional oncogenesis has been observed in multiple trials since the early 2000s. The US FDA wanted a formal regulatory mechanism to receive data on delayed adverse events and to provide guidance on the types of data to collect.

The goal was and still is to understand the long-term risks of gene therapy products. Some of the early discussions to help build policy included three public FDA advisory committee meetings that occurred between 2000 and 2001. There was also a public workshop at the 2001 annual meeting of the American Society of Gene Therapy. Ultimately, this led to the first guidance on LTFU, which was finalized in 2006.

The FDA's guidance has been and still is risk-based, and is based on a number of factors such as persistence of the vector, long-term gene expression, and integration potential. These factors can be based on scientific knowledge, clinical data, and/or preclinical data. The agency advises that for integrating vectors or products that persist, subjects should be observed for delayed adverse events for as long as 15 years following exposure. LTFU observations should include a minimum of 5 years of in-person examinations with lab testing if appropriate, followed by 10 years of annual queries of study subjects either in-person or by questionnaire.

Questions can include whether subjects have new malignancies, or new incidents of neurological disorders, autoimmune disorders, hematologic disorders, and more. Integrating vectors have some more specific long-term lab testing recommendations to assess integration patterns, including appropriate surrogate cells—such as peripheral blood mononuclear cells—for up to 15 years in order to detect monoclonal outgrowths.

In terms of policy evolution, there is an updated guidance that was released and finalized in 2020. This now includes recommendations for newer products such as gene editing products, with LTFU recommendations for those types of products for up to 15 years.

There is also some discussion on products that have always been considered low risk. There is now more definitive language, which I think is very helpful, including: "clinical data from LTFU observations of subjects that have received plasmids, poxvirus, adenovirus, and AAV in clinical trials conducted since 2006 further supports the assessment of lower risk of these gene therapy products". There has been a downgrading of these products, but again, one of the primary drivers of LTFU is persistence. For something like AAV, which is a product that can persist for long periods of time, the FDA still recommends up to 5 years of LTFU.

LP: The main three aspects center around potential oncogenesis, immunogenicity, and vector reactivation. Sometimes this may include a retrovirus or lentivirus that may lead to or trigger adverse reactions including secondary tumor formation, as well as contributing to clonal expansion.

In late 2023, there was some secondary T cell malignancies were observed in patients who had received CAR T cell products. The number of patients affected was not high—around 20 cases—but that led us to reflect on what action was needed. What are the stringencies that we need to apply, and what are the observations? This area is very dynamic and complex, including clinical data, preclinical data, and investigator observations, and some of the nuances may not be clear.

The policies and guidance are clear, but this remains a dynamic and evolving area. Opportunities to speak on these issues can really help to create mutual engagement with different communities, the scientific industry, clinicians and sites, in order to define boundaries and minimum criteria.

Q What would you define as the key challenges posed by LTFU studies?

DT: There are challenges for early and late stages. The greatest challenges I've seen are for some of the older legacy programs, and understanding when you can discontinue LTFU. I've had many clients that are doing a fantastic job and doing their best to keep LTFU studies going well after their primary study has ended, but there have been very few subjects remaining, due to either disease progression or dropouts for personal reasons. More importantly, many of these companies have data showing that there has been no product persisting for years. In these cases, it's reasonable to ask for FDA to discontinue LTFU so you can inactivate or withdraw your IND.

At the earlier stages, you have to understand what the requirements are. It's also an opportunity to collect additional information on long-term efficacy and duration of effect, and to get a good, streamlined protocol in place so you can gather optimal data for regulatory requirements and any additional data you might want to collect.

KW: Most gene therapies are a once-and-done therapy and the drug persists for a long time. Unlike small molecules, where compliance is focused on continued dosing, with gene therapy the compliance problem is following the study protocol for a long period of time and tracking these patients. Making that protocol as easy as possible to comply with is a key issue for gene therapies.

ED: It comes down to ensuring that you are pre-planning for your LTFU during clinical trial development. This allows you to take in all the considerations. Are you going to have multiple studies that will be rolling in? Could you utilize an umbrella protocol, or will you have numerous indications within a therapy where you could implement a master protocol? These are all efficient methods, but you have to think about how you are going to execute this so that you have your LTFU study up and running, ready to roll patients over when you need to.

"When considering survival and follow-up in the cell and gene space, it's important to firstly remember that some patients may be undergoing several lines of therapies." — Luis Arthur Pelloso

Going back to the points of retention, making the study very easy to participate in is a key piece. These patients are probably going through various research studies. They could have been in a natural history or a disease registry going into a clinical trial, and now a LTFU. Think about study fatigue and how that's affecting your patients. From a very long-term perspective, what are our options to perhaps decentralize and bring the study to the patient? At the moment with these newer therapies, many are restricted by the location of the site that can perform the treatment or the production of the treatment. So if patients are traveling, how far are they traveling? Can we provide them with travel services? Additionally, don't forget about the toll taken on a caregiver in terms of planning.

There are many things that I perceive as operational challenges, but there are opportunities for us to come up with solutions and alleviate burden on both the site and the patient. The aim is to come up with solutions that are going to bring the study to the patient when possible. We also have to think about this 10 or 15 years from now. The solutions and technology that you put in place now should ideally be able to evolve over time, and remain flexible, in order to achieve what you need to.

LP: Considering the patient journey, it likely takes a while for a patient to feel engaged. Usually when a participant is attending a cell or gene therapy trial, they are very willing to engage with the opportunity to treat their disease. In the early stage of toxicities, if you think about a cell therapy that may be associated with cytokine release syndrome and neurotoxicity, those early stages of toxicities are relatively manageable in the hospital setting. When it comes time to do a LTFU, sometimes patient adherence and motivation is an issue. Our aim is to make this journey feasible and tenable.

Certain patients may also switch between lines of therapy. For example, an oncology patient may undergo multiple lines of chemotherapy or immune-mediated therapy. Often there is a risk of this patient developing a secondary malignancy not associated with the cell therapy product—but it is mandatory for the sponsor who is running the trial to also collect this data. How do we dissect this information, and what is tangible and doable at the patient level?

Q What are the key regulatory considerations when planning and executing a LTFU study in this space, and what would your key advice be to ensure regulatory compliance?

ED: From an operational standpoint, when you're thinking about the regulations, it goes back to the pre-planning. We know that regulatory agencies are going to require LTFU studies for cell and gene therapies. Guidance recommends having a separate LTFU protocol. Again, very early on in your clinical development you should be thinking about what your design is going to look like. Obtain feedback from the agencies as early as possible.

Another piece is when you are thinking about your schedule of events, consider what impact this is going to have on the site and the patient. What are you trying to collect? Is it everything but the kitchen sink? We shouldn't do that! We should instead be very specific about the critical variables that we are going to capture.

You want to make sure you are capturing information that is going to support you down the road (i.e., safety outcomes, clinical outcomes, information for payers and reimbursement). Again, think of that burden. You can always reach out to the patients to get their perspective in various ways (outcomes that are important to them versus clinical outcomes), and find out

how the schedule of events might impact them. Again, this is ultimately going to help you with the design and overall compliance over time.

DT: Novel products are not well covered in existing guidelines, so it's always important to talk to the FDA early—pre-IND ideally—about your requirements. You can't just say, "Here's my product, I've modified it, so I think it's safer. What do you think?" It has to be, "Here's my data, here's my plan preclinically to show that it doesn't persist anymore or doesn't integrate anymore." You have to develop a robust scientific justification and preclinical plan, so the FDA can assess for themselves what data you have, and what your plan is to show that LTFU isn't needed, if that is the case, so they can give you good advice. There always seem to be gray areas, and it's good to plan this all out early.

One thing that always seems to come up is older programs and the question of when you can discontinue. There are a lot of companies that are doing a good—perhaps too good—job keeping programs open. However, for some it has been a very long time since they have shown any product persistence and they are no longer collecting any useful data.

KW: It's important to keep in mind that the purpose is to understand the long-term risks. If an event happens and you don't have the appropriate assessments and samples available to assess that risk and then mitigate against it in the future, then you're probably falling short. The whole point is helping us to understand what's going on with these therapies over the long-term. If we do have the right assessments and samples available to assess those risks, then we can help the whole field progress.

Q Patient experience is another crucial consideration with LFTU. Why is it so important to understand and consider the patient journey when planning these studies?

LP: When considering survival and follow-up in the cell and gene space, it's important to firstly remember that some patients may be undergoing several lines of therapies. Secondly, there is the risk of additional medical issues or ongoing issues because of their disease, that may pose extra challenges or burdens for the patient.

Some patients may find it challenging to adhere to LTFU due to the nature of their disease. Sometimes they will be unable to come to the site. As we have discussed, we can consider solutions such as telemedicine that can potentially facilitate follow up, and alleviate burdens on the patient.

Another challenge is impaired cognitive function. Some patients in receipt of a heavy doses of chemotherapy may have some cumulative damage, and therefore, some may have some cognitive

"It's important to keep in mind that the purpose [of a LTFU study] is to understand the long-term risks. If an event happens and you don't have the appropriate assessments and samples available to assess that risk and then mitigate against it in the future, then you're probably falling short."
— Keith Wonnacott

impairment. How can patients comply if they have difficulty understanding the long-term assessments? The patient may be ok during the trial itself, but as patients age, this can become challenging.

We may need to consider what the minimal regulatory requirements are for safety, and consider how to integrate dedicated collaborative teams encompassing the clinical, medical, and regulatory stakeholders, working in full conjunction with the sponsors to collect data to meet the minimum criteria.

ED: From my perspective, clearly, retention is key. That is a major focus for us operationally when we are looking at a LTFU study. As Luis mentioned, this entails thinking about the patient's journey, and how their indication impacts their daily lives. How would that be impacted by the assessments that we're trying to collect, and the timing of collection? Taking all of that into consideration and how that is impacting the patient, but also their caregivers and their families, is crucial.

We have to ensure that the solutions that we are putting in place to retain these individuals are truly going to be beneficial to them and not over-engineer it. Again, we have travel services, concierge, and reimbursement that can help. That alone alleviates some stress from a caregiver's perspective. Then we have other things like wearables to help us collect data more efficiently.

Over time, the hope is that these individuals are going to get better. They are going to want to live their life and won't want to constantly be a research subject. We want to make it easy for them. We must allow for flexible through any of those life changes that may occur, whether they need to move, go to school, get married, all those wonderful things that could happen in the future!

KW: It's important to help the investigators and the patients understand the 'what' and the 'why' before they start. If they understand, it will help them to implement the protocols. As discussed, we should make the protocols as easy as possible to adhere to. Finally, I would add from the corporate side, be thoughtful about how you design things; whether it's one protocol that includes both the treatment phase and the LTFU phase, or two protocols. There are pros and cons to the different approaches. If you get stuck with separate protocols and separate contracts and responsibilities, it may not, in the end, be what you wanted. Think that through carefully.

DT: I would just circle back to the regulatory requirement part. Regulatory agencies such as the FDA certainly understand the challenges with patient retention on these LTFU studies.

The intent is that LTFU is an upfront commitment by the IND sponsor. Patients have to be consented upfront that they will be asked to participate in LTFU, but they certainly have the right to discontinue at any stage. You do your best to try to explain how important it is to understand long-term risk, both for the trial itself and for the larger field. But as was already mentioned, no one wants to be a research subject for 15 years. They want to move on with their lives. It's a difficult balance. However on the regulatory side, the FDA does understand the challenges, and it's really just an upfront commitment by the sponsor and by the company.

Q Could the panel share their predictions on what LTFU studies will look like in future, as the reach of clinical and commercial CGT products continues to expand?

KW: To start with, let's just be clear that it's all about risk. As we learn more, and we understand that the risk is either increasing or decreasing based on the knowledge gained, the

"From an operational standpoint, we have outlined various ways that we can bring the study to the patient. We have so many options available to us now, but I'm very eager to see where we go next in terms of new and innovative solutions." — Elizabeth Donahue

follow-up requirements will adjust accordingly. We have already seen that with AAV requirements going down to 5 years. I think we'll continue to see that as we learn about the risks.

I think the other thing that will evolve—and we've seen this already—is that when we learn that certain vectors have risks associated with them, those vectors will either be improved upon or eliminated from clinical development programs. Finally, the way that we collect LTFU data will evolve. Electronic records will continue to make it easier to follow up patients, and we're already seeing the use of registries and other forms of real-world data.

ED: Keith really hit the nail on the head there—understanding the need to evaluate the data over time, and being able to pivot based on the knowledge gained, is going to be key. From an operational standpoint, we have outlined various ways that we can bring the study to the patient. We have so many options available to us now, but I'm very eager to see where we go next in terms of new and innovative solutions.

DT: As more knowledge is gained and products and vectors improve in terms of their safety profiles, I think any delayed adverse events are going to be increasingly rare. Trials seem to be getting shorter and shorter, and hopefully in the future, more emphasis will be placed on post-marketing studies to gain LTFU knowledge. Again, hopefully we'll have a good knowledge base to understand that certain safety risks are going to be very low frequency and we can move them to post-marketing.

LP: The knowledge we have gained in the last decade or two in assessing safety events is giving us some useful data to act upon. We also can look at digital technologies and artificial intelligence methods to aid in collecting data robustly. This can help to amplify the way in which we are fulfilling long-term safety requirements.

Some safety events will continue to occur no matter what, but the goal should be for unusual safety events, and those severe enough to shut down a program, to be reduced. It is up to us, with the aid of emerging technologies, to contribute to increased safety and a reduction in the burden upon patients when taking part in longer-term studies.

Just a few years ago, none of us would think twice about driving to a local pizzeria to get a pizza. Fast forward to today, and we're using our smartphones to have someone deliver it to us. As the way we collect data for these studies evolves, so should the way we interact with patients—with a goal of making simple, accessible roadmaps and pathways for patients and their families. In turn, the more real-world evidence we can collect, the more precise and useful our data will be.

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LUIS PELLOSO is the Executive Medical Director in Medical and Scientific Services within Pharmacovigilance at PPD, Wilmington, NC, USA. Currently Pelloso leads the Oncology Medical Monitoring globally. He has served as an oversight therapeutic MM lead for hematology-oncology medical monitoring, as a Global Medical Director for more than 50 Phase I–III trials, provided strategic vision and business direction within the department for MM and PV services and cross-functional within hematology-oncology and rare diseases for 13 years. Pelloso received his MD in 1997 from the Faculdade de Medicina do ABC, Santo André, Brazil. He did his residency in Internal Medicine in Federal University of Sao Paulo, Brazil (UNIFESP), where he also did his Fellowship in Hematology and received a PhD in Medicine in 2005.

DANIEL TAKEFMAN is the Principal of Takefman Gene Therapy Advisors, Philadelphia, PA, USA, since March 2020. He provides expert regulatory advice for the development and commercialization of cell and gene therapies. Takefman also assists in due diligence assessments of gene therapy assets for venture capital firms and is a member of multiple scientific advisory boards. Previously, Takefman was SVP and Head of Regulatory Affairs at Spark Therapeutics for 5 years. At Spark, Takefman supervised the submission through to approval of the FDA and EMA Luxturna® marketing applications. He also supervised the regulatory process for multiple AAV-based investigation products, including two additional breakthrough designation products: SPK-9001 for the treatment of hemophilia B (marketing applications currently under review by FDA and EMA) and SPK-8011 for the treatment of hemophilia A. Takefman joined the FDA Division of Cellular and Gene Therapy in 1999 as a Postdoctoral Fellow. Takefman held multiple positions during his 15 year career at the FDA, ultimately becoming Chief of the Gene Therapy Branch. He supervised the CMC review process for all gene therapy products and for a variety of therapeutic vaccine products. Takefman holds a PhD in Immunology from Rush University, Chicago, IL, USA, and a BS in Microbiology from the University of Iowa.

KEITH WONNACOTT joined LEXEO Therapeutics, New York, NY, USA, as the Vice President of Regulatory Affairs in November 2021. Prior to joining LEXEO, Wonnacott was an Executive Director of Regulatory Affairs at Pfizer, where he had responsibility for gene therapies within the Rare Disease business unit. He provided guidance on regulatory strategy and led regulatory policy for the gene therapy portfolio, which included advancing three late-stage programs into Phase III clinical trials. Wonnacott also worked at Novartis, where he was a Director of Regulatory CMC and led the development of Module 3 for the Kymriah BLA, which became the first gene therapy product to receive FDA approval in 2017. Wonnacott also held roles of increasing responsibility at the FDA at the start of his career. He spent 13 years in the Division of Cell and Gene Therapies at the FDA, the last ten of which were as Chief of the Cellular Therapies Branch. In total, Wonnacott has spent his entire career of two decades working in cell and gene therapy. He is a respected leader in the field, serving in many roles on trade association and society committees. He recently completed a term as the Chair of the Regulatory Affairs Committee for the American Society of Gene and Cell Therapy. Wonnacott has spoken and published extensively on topics related to the regulation of cell and gene therapies. Wonnacott received his PhD from the Pennsylvania State University, PA, USA, and his undergraduate degree from Brigham Young University.

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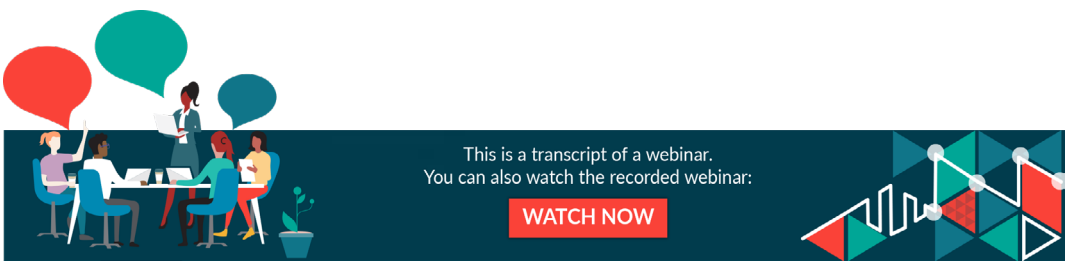

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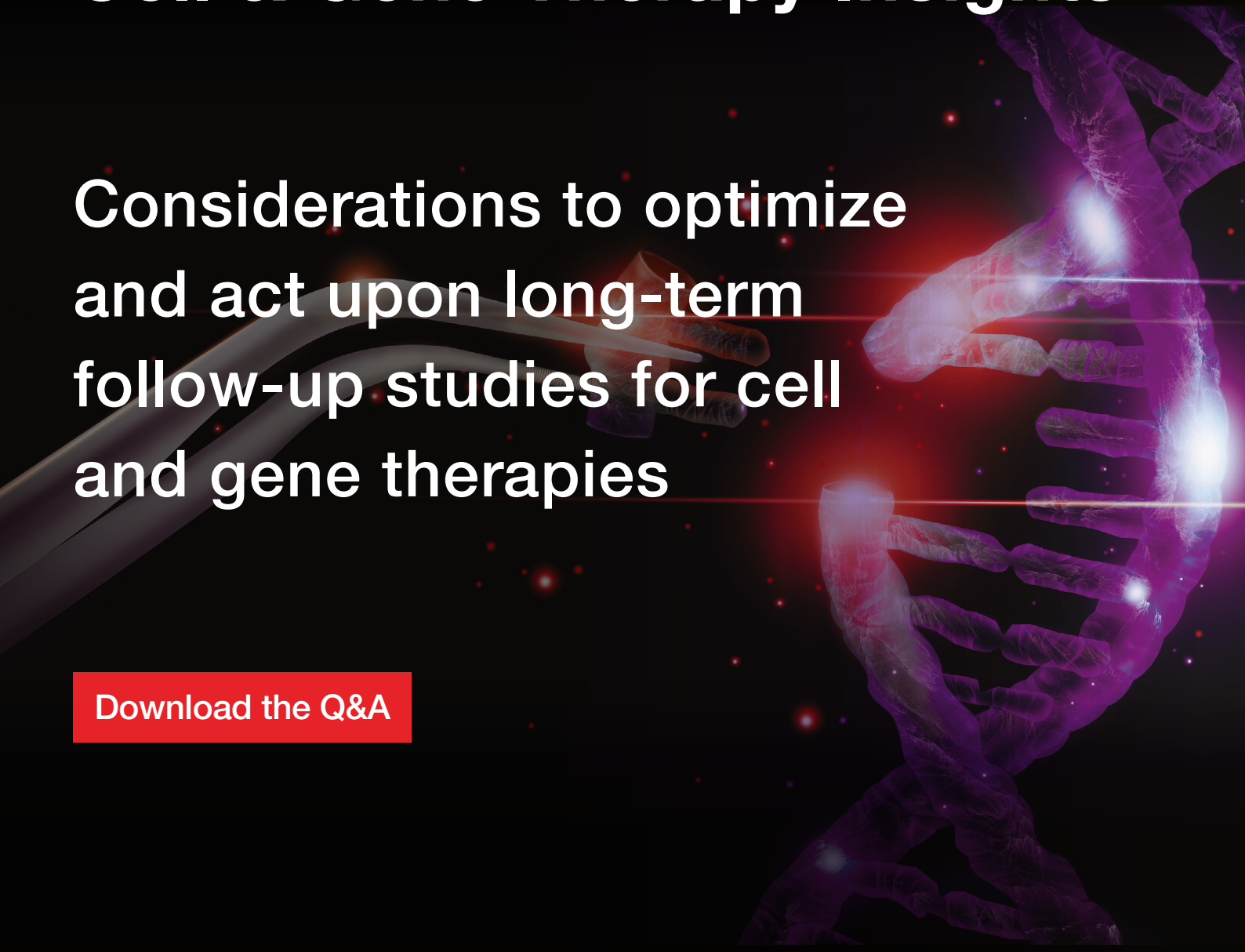

Considerations to optimize and act upon long-term follow-up studies for cell and gene therapies

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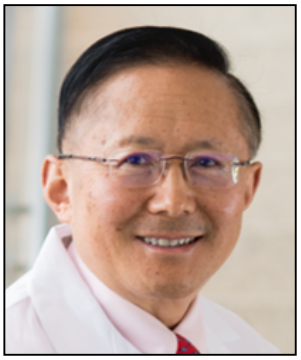
CELL & GENE THERAPY INSIGHTS

EXPERT ROUNDTABLE

Unveiling the critical pathways in AAV potency evaluation and future trends in gene therapy

David Rangel, Guangping Gao, and Nathalie Clement


In this expert roundtable, three experienced professionals from the gene therapy industry discuss the critical evaluation of AAV potency and its impact on the industry's advancements. The panelists share insights on CMC evaluation, varied assay development based on therapy mechanisms, and platform evolution.

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Q Could you provide an overview of the critical aspects involved in evaluating potency for AAV products, and how they have contributed to the advancements of gene therapies?

GG: Potency assays are a key aspect of gene therapy product evaluation. Currently, the potency assays used are varied and depend on the mechanism of action of the product. For instance, assays for an enzyme, secreted protein, or structure will vary greatly. In my experience, it is crucial to evaluate the therapeutic outcome following *in vivo* assay, additionally, *in vitro* assays are essential to characterize the potency of the gene therapy vectors.

DR: In terms of process, I suspect that the Q14 ICH revision, which offers more guidance on the analytical method life cycle and evaluating robustness and performance, is related to potency. This is due to many companies overlooking potency assessments for their products due to their inherent complexity. Hence, that is likely why the new revision puts more emphasis on how assays are developed and how their evolution is tracked during development and use.

NC: Looking at the CMC side, AAV potency evaluation is crucial. This evaluation should be started as early as possible as it can be critical in determining comparability as the product development matures. The guidance on comparability studies has also shown that potency is a key parameter. It becomes a signature throughout the product development journey, as it will be exposed to changes at many different levels. Understanding the significance of potency at any given time in your CMC development is going to be crucial.

Q Considering the complexities of changing serotypes and the importance of downstream analytics, how would you approach developing assays for CMC purposes to ensure flexibility and reliability in AAV product manufacturing?

DR: To address this question, I would heavily rely on platform analytics, an area that has been gaining significant attention lately. Each company has its analytics platform they are looking to produce and offer to clients; however, caution should be taken with these. Time management and experiments should be included in these platform analytics to allow the same amount of client-specific experimentation we have previously had when developing a client-specific assay. The goal is to leverage institutional knowledge and the platform to customize specific activities for each client-specific material. This approach will enhance our overall culmination of knowledge related to a specific analysis, enabling us to tell a clearer story for a client's product during their filing process.

"...it is crucial to understand the final product as quickly as possible, especially with regard to serotype."

NC: I want to further emphasize the importance of David's point, which is often underestimated: the time taken to develop these assays. In other words, it is crucial to understand the final product as quickly as possible, especially with regard to serotype. If you are opting to use an uncommon serotype, perhaps due to IP considerations, you need to ensure you are investing resources. This is not only for purification and downstream but also for assay characterization. Not every serotype has all the tools already developed, such as antibodies or serotype-specific tools, thus choosing a serotype can also affect and impact timelines in assay development.

GG: Due to the differences in serotype, infectivity, and other assays, indicator cell lines will be key in AAV product manufacturing. However, choosing the right indicator cell line to get a biological readout will vary depending on the serotypes.

DR: As there are few potency assays, several companies rely on infectivity. I have repeatedly seen a theme where platformed infectivity assays do not necessarily use an optimal cell line for their infectivity for their unique serotypes. This is extremely relevant and should be considered moving forward.

Q How would you employ sequencing approaches to characterize AAV preparations effectively?

GG: Due to the spatial structures of AAV's immediate inverted terminal structures, the secondary structure prevents the classic Sanger sequencing method from reading the entire AAV genome. Given the nature of these inverted terminal repeats (ITRs) as replication origins and packaging signals, particularly in single-strand vectors, replication becomes a key step in AAV production during *in vivo* application or the production process.

The presence of the secondary structure within the gene of interest or expression cassette, such as CG-rich regions like a CAG promoter, or when incorporating gene silencing molecules such as small hairpin RNA or guide RNA for gene editing, signifies the prevention of DNA primaries from reading through. For that reason, template-switching events may occur during replication due to strong signal structures, causing replication to halt until the next end of the ITR, resulting in truncated products in various forms.

This has been a recurring issue depending on vector design. Our group initiated AAV genome population sequencing to address this concern several years ago. We extensively reviewed such

secondary structures and heterogeneity of AAV gene therapy products. Notably, these truncation events, even within empty capsids, lead to the packaging of ITRs into what are known as 'empty vectors'.

All those aiming to be the best in the field will undoubtedly impact the potency of vectors—without the necessary expression cassette, potency is compromised. Secondly, there is a concern for genome stability. Without a clear idea of whether the vector is truncated, its genetic fitness upon *in vivo* application remains uncertain. Thirdly, if a significant number of empty particles remain, with ITRs packaged in, there is the potential for a response given the enrichment of CpG motifs in ITRs.

For this reason, I believe characterizing products with sequencing technology capable of reading through the entire genome from the five-prime to the three-prime ITRs is crucial, particularly with advanced methods like smart sequencing or nanopore sequencing. More attention is being paid to this area of product characterization, evident from the discussions at the American Society of Gene and Cell Therapy this year, where several companies are exploring product characterization by long-read sequencing. While this currently impacts product characterization assays, the longer-term implications on product release are yet to be fully understood.

NC: From a CMC perspective, which nicely ties into Guangping's previous statement, next-generation sequencing (NGS) remains a crucial characterization assay. However, it is important to note that it is not part of the release certificate of analysis. This is primarily due to the current challenges to set specifications and determine what qualifies a product for release. The complexity of AAV drug products is greatly significant, as revealed by NGS.

However, while NGS unveils heterogeneity and complexity, it also opens a Pandora's box. When detected, anomalies must be explained and defined as they could impact clinical safety and efficacy. NGS is an undeniably powerful tool, which will hopefully have the potential to enhance release specifications. However, we must tread carefully as we are still in the process of extracting information that could impact product release, and there remains uncertainty on how to decipher this information.

GG: There are a few challenging aspects to understanding sequencing outcomes and product potency. Firstly, AAV exhibits a robust recombinogenic nature. Upon *in vivo* administration, the genome may undergo truncation, resulting in various fragmented forms. Due to the recombination, these fragments could potentially form intact expression cassettes, thereby partially rescuing transgene product expression.

Secondly, while we understand the product sequence, establishing a correlation between sequence properties and vector potency remains elusive. Without these correlations, utilizing sequencing as product release assays remains a challenge until we accumulate more data. Our laboratory has extensively pursued this avenue, conducting over 100 next-generation or long-read sequencing analyses of diverse lots where we are trying to establish these connections. Concurrently, we are employing deep machine learning to gain insights from these extensive

"...if an intermediate fragment remains functional, should it be considered full, and do we require potency data to make this determination?"

reads, aiming to discern the causes of truncation or partial genomes. Hopefully, our AI algorithms will guide us in designing vector genomes effectively.

DR: Currently, several tools are available that can separate these intermediates, such as analytical ultracentrifugation. Although there has been an emphasis on characterizing the intermediates, the focus remains on potency assessment. While these fragments can be sequenced, their functionality remains unclear. Clients are having further difficulty in establishing specifications for empty and full vectors, especially determining what constitutes 'full'. We can provide guidance here, but if an intermediate fragment remains functional, should it be considered full, and do we require potency data to make this determination?

Many clients are opting to specify non-empty vectors, leveraging potency assays to establish better correlations between potency and empty versus full vectors, even when partial intermediates are included. The need for potency assessment persists as we look to characterize these products.

Q With the emphasis on assessing analytical compatibility across diverse AAV products, how would you propose addressing the need for standardized methods?

DR: Besides restating the importance of having a flexible platform capable of assimilating new and client-specific material, I would like to emphasize a crucial point about analytical comparability. Many clients are employing a signature AAV production platform from a specific vendor while simultaneously utilizing assays from that same vendor that the client does not own.

One common challenge I often see is clients transitioning between CMCs while simultaneously adopting new platforms and analytics. This makes it difficult to evaluate the comparability of their production process separately from their product and analytics, as they are often changing all three elements concurrently. My advice would be to collect as many samples as possible from the outset and store them in a freezer. Just because you are on one particular production platform now does not mean you will stick with this for the next 5 years.

Clients often delay switching platforms or try to remain with a company for as long as possible. However, by the time they decide to switch, they are constrained because they can only move from one manufacturing and analytics suite to another, while also lacking the historical

samples needed to show comparability. This can cause resistance to change, with individuals being reluctant to make much-needed changes. In an industry where a product's lifecycle could span 5 years, that period represents a significant period of evolution.

Over these years, numerous advancements could emerge to enhance yields for clients and reduce residuals. However, there is a notable reluctance to update filings which can hinder the adoption of these process improvements. I have seen individuals trying to maintain comparability with past practices rather than embracing the truth revealed by their data. They seem fixated on replicating the answers obtained years ago, regardless of their accuracy.

NC: It is not just clients or sponsors who may switch manufacturers or testing groups, but within the same group, we can change testing, assay development, or introduce a new instrument, all proving more successful than the previous versions. The landscape is dynamic, and such changes are likely to persist even though there have been efforts to standardize assays themselves, which would be a significant advancement.

One part of the solution, albeit a work in progress, involves incorporating controls or reference standards that could unify production batches and facilitate comparison with other drug products in clinical settings. While this might not be the focus of today's discussion, organizations such as the National Institute of Standardization and Technology are working on developing reference standards for AAV. However, the widespread incorporation of these standards by all groups and their integration into assays remains a challenge.

Another frustration for gene therapy developers is the repetitive process of reinventing the wheel for each new product. Despite the great amount of information that has been gained throughout numerous clinical trials at the FDA level, there is a lack of consistency in release assays and testing methods among sponsors. This further contributes to a lack of prediction on product safety.

This inconsistency extends to critical quality attributes such as full and empty capsids, which are often tested by a single vendor or group, making it difficult to extrapolate how one drug product compares to another. In an ideal scenario, standardized assays and platforms would be universally adopted, allowing for direct comparison of certificates of analysis from different drug products. Unfortunately, we are not at that stage yet. This has been a limitation in the pace of developing better drug products for clinical use.

Q What strategies would you suggest to advance our understanding and predictive capabilities in assessing the potency of future AAV products?

NC: As mentioned earlier, potency is going to be key to success throughout the drug product development. However, it has posed challenges for those involved due to several factors. First, there is no one-size-fits-all approach due to the variability in serotypes. Second, potency assays are primarily *in vitro* cell-based, and it is important to acknowledge that these

pose limitations. It is somewhat restrictive to assume that a drug product will behave similarly *in vitro* as it would in a human. The relationship between *in vitro* potency assays and *in vivo* efficacy requires further development and understanding. Additionally, the diversity in serotypes among individuals impacts comparability across various products.

GG: Potency assays are inherently complex. Each gene therapy product operates through a unique mechanism of action. Consequently, some products, such as those involving secreted substances, may present simpler assessment challenges. Other products, such as those involving enzymatic reactions, can prove more intricate. Considering scenarios like gene replacement or gene silencing for enzymes, it is difficult to effectively measure the functionality of structural proteins.

It is plausible that such assessments must be based on an analysis of cellular or tissue morphology. From the perspective of scientists engaged in academia and basic research, tackling these complexities can be daunting. At the same time, industry groups appear to adopt a more innovative approach, employing a range of qualitative and quantitative assays, including mass spectrophotometry, to discern minor or significant differences.

Having engaged with numerous industry professionals on potency assays, it is evident that a standardized approach is needed to gauge therapeutic outcomes, albeit tailored to each specific product. This is reflective of the early days of gene therapy development, where *in vivo* data from animal models served as the primary measure of therapeutic efficacy. This further shows that reliance on such methods can lead to issues of reliability and reproducibility. Therefore, the shift towards *in vitro* or product-release plaque assays signifies a critical evolution in ensuring consistency and quality control.

DR: *In vitro* potency is complex, primarily due to the significant time and financial investments required to develop platforms capable of replicating diseases for *in vitro* evaluation. Currently, it seems the industry remains undecided on whether such endeavors warrant effort and resources. This uncertainty is closely tied to the debate surrounding the utility of non-human primate (NHP) models. Despite advancements, NHPs are still utilized in preclinical testing before human trials.

I am not yet convinced that any compelling data exists to necessitate the development of potency assays. The debate between *in vitro*, *in vivo*, and human-based testing methodologies lacks clear correlations. Until we commit to exploring this further and allocating resources accordingly, clients may continue to face challenges.

Many stakeholders that I speak with regularly express reluctance toward investing in potency assays because they anticipate stringent requirements for compliance. They know that upon establishing a specification, they will be bound by the International Council for Harmonization parameters, requiring full assay validation before advancing to Phase 3 or process performance qualification, and are uncertain about both feasibility and funding.

There is a lot of ground to cover in understanding this topic within our industry. It would be beneficial if we could develop a platform that renders non-human models irrelevant, ultimately leading to improved outcomes.

NC: I completely agree with Davids's skepticism regarding the utility of developing potency assays as a comprehensive predictor of human outcomes. We all recognize the limitations and I want to emphasize the complexity of developing a potency assay. It is crucial to remember that it is a case-by-case scenario. It heavily depends on the sponsor's gene of interest and the serotype involved.

Given this complexity and the need for a tailored approach, there are two stages for the development of potency assays that are phase appropriate. In Phase 1, theoretically, demonstrating the activity of your gene product is not necessary. Instead, the expression must be shown. As you mature to Phase 3 and the Biologics License Application, potency assays capable of measuring activity become essential.

I would like to challenge the notion that any activity assay can accurately predict or measure in vivo outcomes. From my perspective, potency assays serve more as a comparability tool throughout the CMC journey, especially from batch to batch. Even minor changes, such as alterations in serotype, may necessitate monitoring to track the evolution of our drug product.

GG: David raised an interesting point about product release characterization assays. I firmly believe in the importance of utilizing NHPs as a crucial link between mouse studies and human applications. However, while NHPs have proven invaluable in assessing toxicity and biodistribution, I am uncertain how we can effectively evaluate product potency or activity beyond expression if we are fortunate enough to observe secretion or expression through techniques like western blotting or histology. I sincerely hope that we can develop more informative assays for assessing the potency of the vector.

Q Looking to the future of AAV-based gene therapies, what would be your predictions for future trends and challenges—and how do you envision these shaping the landscape?

GG: Firstly, the current range of available delivery platforms, specifically AAV capsids, is quite limited. We are in search of more potent vectors with specific tropism. Fortunately, the field has been diligently working towards this goal. We have witnessed encouraging developments from various companies developing cross-blood-brain-barrier serotypes for use in the central nervous system and academic activity aiming for systemic delivery of AAV to target muscles. These developments are hopeful indicators that these vectors will soon find their place in the clinical testing pipeline.

Secondly, manufacturing poses a significant challenge. Irrespective of the current methods and processes employed, manufacturing remains costly and is often accompanied by QC constraints, such as achieving optimal empty-to-full ratios. We are aware that an excess of empty capsids can trigger immune responses in human patients.

"We are entering a stage like the early days of tissue transplantation, where host responses, particularly at high systemic doses, pose challenges."

We have also delved into discussions on vector genome truncation. Consider a scenario where only 30–50% of vector preparations contain fully functional genomes as intended, what steps can then be taken to improve the quality and yield, especially with high costs?

Thirdly, we face the question of immunogenicity and toxicity. Achieving broad vector genome distribution, efficient gene transfer, and adequate potency to target toxic cells necessitates the use of high systemic doses. However, administering high systemic doses can cause a lot of problems. These include innate responses such as Toll-like receptor (TLR) 9 and TLR2 activation, and component activation in the early stages. Subsequently, there is potential for adaptive responses to both the capsids and the transgene product. This further complicates matters due to the added layer of complexity introduced by adaptive responses.

At this point, we often pin our hopes on the notion that a single injection of AAV can yield long-lasting effects. However, achieving this remains a challenge. The necessity for re-dosing depends on various factors such as the age of treatment initiation and specific target tissue, such as the liver versus the central nervous system, each yielding distinct outcomes. It is possible that soon, the need for re-dosing may become apparent. However, overcoming the barrier posed by re-dosing presents another challenge. Addressing early-stage innate responses and complement activation is crucial but also comes with difficulties.

We are entering a stage like the early days of tissue transplantation, where host responses, particularly at high systemic doses, pose challenges. Additionally, most current gene therapies remain unregulated, often relying on strong, ubiquitous promoters. This raises concerns regarding safety and durability. It is plausible that in due course, regulating expression, whether via tissue-specific, pharmacologic, or other methods, may become a prerequisite for safe and enduring therapy.

DR: My perspective leans more towards the mechanical aspects. As the industry settles on a more unified downstream and upstream purification process, we will witness a maturation like what was seen with antibodies. This evolution will lead to broader usage and specific in-process controls, providing practitioners with greater certainty and flexibility to implement process modifications.

It becomes more clearly defined how to make improvements over time when there is a better understanding of the process. People are going to be more open to incorporating newer technologies and applying lessons learned into longstanding processes. Also, as we deepen our understanding of these platforms and conduct comparability studies on potency, updating processes will likely become more streamlined and cost-effective. This, in turn, will facilitate the delivery of superior products compared to the initial iterations.

NC: The future, as described by Guangping and David, aligns closely with my perspective. However, the only little subtlety from David's view is that I do not believe the field has fully settled on one platform, particularly in the upstream processes. I am uncertain whether to find this trend enjoyable or frustrating as it does add complexity, especially when comparing drug products across different sponsors.

Depending on the chosen upstream platform, critical quality parameters and attributes of an AAV drug product may vary significantly. As Guangping mentioned earlier, the heterogeneity of sequences, including full, partial, and empty components, as well as potency, may be impacted by the upstream platform. Currently, three main platforms are in use: transfection in human HEK-293 cells, producer cell lines in human HEK-293, and the baculovirus system in insect cells. Despite the additional complexity introduced by these differences, they have not yet been linked to any clinical deficiencies or impacts. I anticipate these three platforms will continue to be utilized for the foreseeable future.

Standardization of the downstream processes and assays is crucial for accurate comparisons. Further, AI is becoming a critical tool in vector sequence development to minimize heterogeneity and enhance the percentage of fully functional sequences from the outset. AI is also facilitating the development of new capsids tailored to specific tropism required for cell types.

Further, automation is gradually becoming integral to manufacturing processes, albeit in its early stages. Over time, automation will enhance robustness across various levels of manufacturing, ensuring predictable outcomes in terms of both quality and quantity. Implementing robotic automated dilution before potency or PCR assays will aid in reducing variability within these assays. I think that we all agree that there is still a lot of work to be done.

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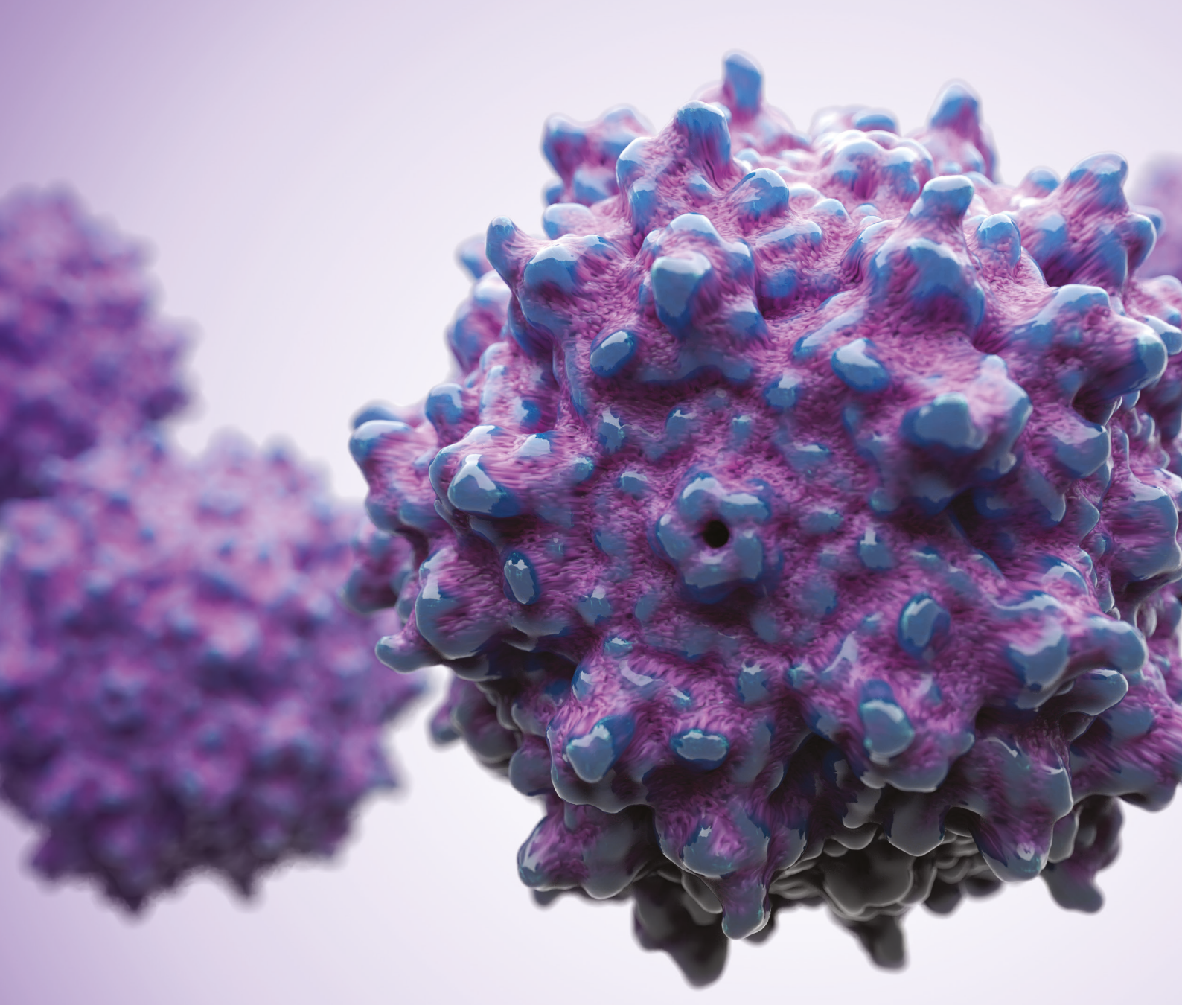

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Cryopreserving CAR-T cells in a novel rigid container maintains their phenotype and function compared to conventional cryobags and cryovials

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CAR-T cell therapies are rapidly emerging as an effective treatment and even cure for malignant cancers. How these therapies are cryopreserved is essential to preserving their cancer killing function and how they are shipped is important in reliably delivering these life-saving treatments to patients. Here, we compared CAR-T cells cryopreserved in a novel, rigid-walled container—the CellSeal® CryoCase™ (CryoCase)—to those cryopreserved in the conventional cryobags and cryovials used in the industry. We found that CAR-T cells can be effectively cryopreserved in the CryoCase using the same controlled rate freezing profiles and methods used for standard cryobags and cryovials. CAR-T cells cryopreserved in the CryoCase maintained cell viability and cell recovery above 85%, similar to CAR-T cells cryopreserved in both cryobags and cryovials. Expression of the chimeric antigen receptor (CAR) on T cells was similar across all cryopreservation containers tested. CAR-T cell phenotype was also comparable across the different cryogenic containers, with no significant difference in the distribution of CD4+ helper T cells and CD8+ cytotoxic T cells, as well as naïve and memory T cells. The CryoCase was also compatible when tested in a fully automated and closed CAR-T manufacturing process, where it provided a robust and easy-to-use solution for product fill and finish, with critical quality attributes identical to CAR-T cells stored in cryobags and cryovials. Collectively, these results offer insight into a novel cryopreservation process and container for CAR-T cells, and explore how the fitness and function of CAR-T cells compares across the different containers that they are cryopreserved and stored in.

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INTRODUCTION

CAR-T cell therapies have revolutionized the treatment of malignant cancers, offering unprecedented efficacy and the potential for lasting remissions. The success of these therapies depends not only on the engineering of potent CAR-T cells, but also on the meticulous processes of cryopreservation and storage that ensure their viability and functionality from the lab to the clinic.

Since 2017, these cell-based immune-oncology products have continued to transition from translational research to approved advanced therapy products in major regulated jurisdictions around the world [1]. Accordingly, the reliance on legacy manufacturing processes, equipment, and components continues to evolve. Products developed specifically to support commercial scale-out of autologous manufacturing processes of cell-based therapeutics continue to be adopted, reducing the manufacturing challenges arising from the use of borrowed technology. For example, in the last few years, several closed-process fill systems have been released to reduce the manufacturing burden of final fill in Grade A spaces and provide more reproducible processes [2]. Notably, one aspect of manufacturing that has not significantly changed is the toolbox of final product containers. Traditional methods employing cryobags and cryovials have been the industry standard. However, cryobags pose limitations such as fragility and difficult-to-identify particulate burden, and cryovials support a limited range of volumes before the freezing profile across the sample becomes meaningfully different. For example, with respect to cryovials, the surface-to-area volume ratio of a 5 mL vial compared to a 50 mL vial results in substantially different freezing kinetics in the center of the sample. Bag fracture during storage or shipping in the frozen state is likely to result in complete product loss or the need to use a potentially contaminated cell suspension. Critically, discarding the product would lead to treatment delays with serious implications for patient outcomes. While there is variability in fracture rates across bag materials and manufacturers, processing has a major impact on physical stability and high rates of fracture can be observed [3]. Fracture represents a significant risk, and mitigation strategies during transport, shipping, and storage are very burdensome. Fracture may rank as one of the most critical product risks to mitigate in many cases. As made clear by recent regulatory actions [4], particulates are an area of focus and containers that allow improved inspection or manufacturing processes could result in a significant improvement in the industry.

This study explores the efficacy of a novel rigid-walled cryogenic container, the CryoCase, designed to overcome the limitations of existing solutions. We compared CAR-T cells cryopreserved in the CryoCase with those stored in conventional cryobags and cryovials, evaluating key parameters such as cell viability, recovery, phenotypic stability, and functional markers post-thaw. Our findings suggest that the CryoCase not only matches the performance of traditional containers, but also offers enhanced durability and flexibility, potentially setting a new standard in the cryopreservation of CAR-T cell therapies.

MATERIALS AND METHODS

CellSeal CryoCase

The CellSeal CryoCase is a rigid container manufactured from cyclic olefin co-polymer

(COC) with injection molded fill and vent tubes manufactured of ethylene-vinyl acetate (EVA). The fill tube is connected to polyvinyl chloride tubes with multiple Luer-type fittings. The fill tube can be connected using tube welding or through existing fittings. The vent line is fitted with a removable 0.22 µm disc filter to allow air escape and an in-line microbial barrier filter that remains for use post-cryopreservation. The container has a maximum recommended fill volume of 75 mL. Fill and cryopreservation are carried out in a vertical orientation, resulting in consistent surface area-to-volume ratios across a range of fill volumes. Comparable cell recovery and viability has been observed in various volumes ranging from 75 mL to less than 20 mL (data on file).

T cell culture, transduction, and harvest: G-Rex® flasks

CD3+ Pan T cells from normal healthy donors were purchased from Charles River Laboratories Cell Solutions (Cat#PB03NC-4). T cells were expanded in G-Rex 100 flasks for 8–9 days until fully confluent, per the manufacturer's recommended protocol [5]. Cells were expanded in TexMACs® GMP medium (Miltenyi Biotec, Cat#170–076–306), supplemented with 100 IU/mL IL-2 (R&D Systems, Cat#202-IL-050). T cells were activated using Human T Cell TransAct™ (Miltenyi Biotec, Cat#130–111–160) at the manufacturer's recommended concentration. One day after activation, T cells were transduced with a third-generation CD19 CAR lentivirus (Creative Biolabs, Cat# VP-CAR-LC69) at a multiplicity of infection (MOI) of 1 per the manufacturer's recommended protocol. After 8–9 days of culture, CAR-T cells were harvested from the G-Rex flasks and then washed and concentrated using a benchtop centrifuge (Sorvall ST). Cells were washed with Plasma-Lyte A (Baxter, Cat#2B2544X) containing 5% (v/v) of human serum albumin (HSA; Akron Bio, Cat#AK8228–0100) and concentrated to a target volume of 30×10^6 viable cells/mL prior to formulation and fill-finish into different containers.

T cell culture, transduction, and harvest: CliniMACS® Prodigy

To simulate a fully closed and automated CAR-T manufacturing process, CAR-T cells were activated, transduced, and expanded in the CliniMACS Prodigy platform (Miltenyi Biotec) according to previously published protocols. Briefly, CD4+ and CD8+ T cells were isolated from a cryopreserved, healthy donor leukopak purchased from Charles River Laboratories Cell Solutions (Cat#PB001CLP-RnD) using GMP CD4 Microbeads (Miltenyi, Cat#200–070–213) and GMP CD8 Microbeads (Miltenyi Biotec, Cat#200–070–215). T cells were cultured in TexMACs GMP medium (Miltenyi Biotec, Cat#170–076–306), supplemented with 100 IU/mL IL-2 (Akron Biotech, Cat#AR1045–0010). T cells were activated using Human T Cell TransAct (Miltenyi Biotec, Cat#200–076–204) at the manufacturer's recommended concentration. One day after activation, T cells were transduced with a third-generation CD19 CAR lentivirus (Creative Biolabs, Cat#VP-CAR-LC69) at an MOI of 1 per the manufacturer's recommended protocol.

After 8 days of culture, T cells were washed and formulated on the CliniMACS Prodigy according to its automated wash protocol and concentrated via the Prodigy to a target volume of 30×10^6 cell/mL prior to final formulation and fill-finish. Cells were washed with Plasma-Lyte A (Baxter, Cat#2B2544X) containing 5% (v/v) of HSA (Akron Biotech, Cat#AK8228–0100).

Formulation, fill-finish, and cryopreservation: G-Rex flasks

CryoStor® CS10 (BioLife Solutions) was added to cells formulated in Plasma-Lyte A with 5% HSA at a 1:1 (volume:volume) ratio

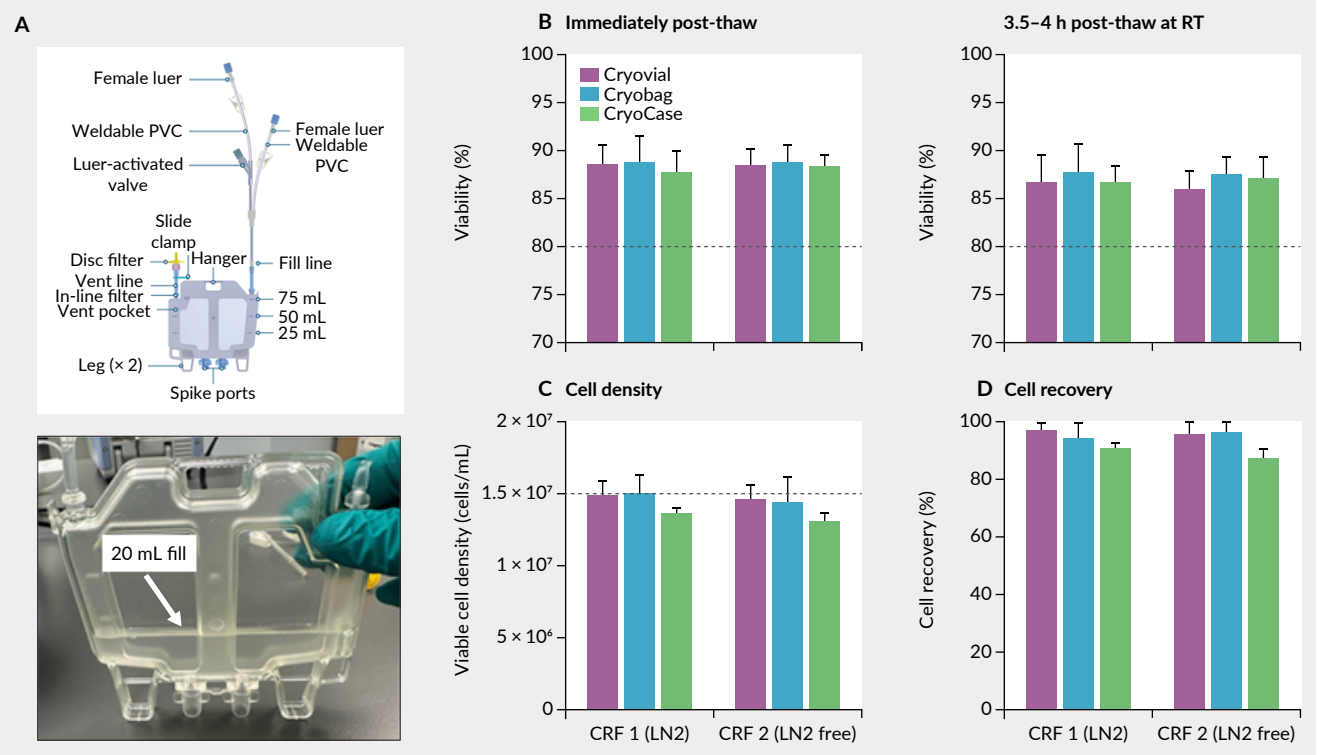
just prior to filling in different cryogenic containers. Final formulation of cell product was therefore 5% DMSO and 2.5% HSA, with a target cell concentration of 15×10^6 viable cells/mL. Formulated cells were then carefully filled into cryobags (Miltenyi Biotec, Cat#200–074–400), cryovials (Corning, Cat#431386), or CryoCases (BioLife Solutions) using a micropipette for small volumes (1 mL or less) or disposable volumetric syringes. Cryovials were filled with 1 mL of formulated CAR-T cells, whereas cryobags and CryoCases were filled with 20 mL or 50 mL of formulated CAR-T cells, as indicated. Filled containers were then transferred to either a liquid nitrogen controlled rate freezer (CRF; ThermoFisher Cryomed™) or a liquid nitrogen-free CRF (Cytiva VIA Freeze Quad) as indicated and underwent controlled rate freezing, according to previously published protocols [6]. Per the manufacturer's recommendations CryoCases were frozen standing up, cryobags were placed in aluminum cassettes and frozen in designated racks standing up, and cryovials were frozen in vial racks provided by the manufacturer of each CRF. Cryopreserved containers were stored in liquid nitrogen until further analysis and use.

Formulation, fill-finish, and cryopreservation: CliniMACS Prodigy

CAR-T cells were formulated and transferred to cryogenic containers (fill-finish) on the CliniMACS Prodigy via a GMP

FIGURE 1

Cell viability and recovery across different cryogenic containers.



(A) Top: Diagram of the novel CryoCase container. Bottom: Photograph of the CryoCase container containing 20 mL of formulated CAR-T cells. (B) Cell viability immediate after thawing each cryogenic container (left) and after 3.5–4 h post-thaw in each cryogenic container at room temperature. (C) Cell density and (D) cell recovery after cryopreservation and controlled thawing in each type of cryogenic container. Cells were cryopreserved in two different CRFs, one which uses liquid nitrogen (LN2 [CRF1]) and one which is liquid nitrogen-free (CRF2). Data shown: mean +/- SD; n = 3 donors. CRF: controlled rate freezers, SD: standard deviation.

Custom Application Program (CAP) developed by Miltenyi Biotec for Charles River Laboratories. Briefly, CryoStor CS10 (BioLife Solutions) was sterile welded onto the CliniMACS Prodigy, and the Prodigy added it to the cells formulated in Plasma-Lyte A with 5% HSA at a 1:1 (volume:volume) ratio just prior to filling in different cryogenic containers. After final formulation and automated mixing of the CAR-T product, 20 mL of cells were transferred via the Prodigy to a cryobag or CryoCase that was sterile welded onto the Prodigy. CAR-T cells were also transferred into a sample bag that was sterile welded onto the Prodigy, and these cells were subsequently aliquoted at 1 mL increments into standard cryovials to simulate samples collected for quality control. Filled containers were then transferred to either a liquid nitrogen CRF (ThermoFisher Cryomed) or a liquid nitrogen-free CRF (Cytiva VIA Freeze Quad) as indicated and underwent controlled rate freezing according to previously published protocols [6]. Per the manufacturer's recommendations, CryoCases were frozen standing up, cryobags were placed in aluminum cassettes and frozen in designated racks standing up, and cryovials were frozen in vial racks provided by the manufacturer of each CRF. Cryopreserved containers were stored in liquid nitrogen until further analysis and use.

Cell thawing, counting, and flow cytometry

Prior to analysis, frozen product containers were thawed at 37°C for 4 min using a water bath. Cell number and viability were assessed using the Via1Cassette™ (ChemoMetec, Cat#941–0012) with the NucleoCounter® NC-200™ (ChemoMetec).

Flow cytometry was performed using the BD FACS Lyric and BD FACSuite software (BD Biosciences). Cells were stained as previously described for the following cell surface markers [7]: CD3 (BioLegend, CA, Cat#317310), CD4 (BioLegend, Cat#344674), CD8a (BioLegend, Cat#344714), CD45RA (BioLegend, Cat#260246), CD19 CAR detection reagent (ACRO Biosystems, Cat#FM3-FY45G0), CD45RO (BioLegend, Cat#304224), CD57 (BioLegend, Cat#393304), CCR7 (BioLegend, Cat#353230), Programmed cell death protein 1 (PD-1; BioLegend, Cat#367428), and 7-AAD (BioLegend, Cat#420403).

Fracture evaluation

To evaluate fracture resistance, the CellSeal CryoCase or cryobags were filled to either 75 mL (CryoCase) or 30 mL (cryobags) and placed into liquid nitrogen for 30 min. Frozen containers were dropped from a height of 2 m onto an epoxy-coated concrete floor.

Data analysis

Data was analyzed and presented using GraphPad Prism software, version 10.X. For statistical comparison, data was analyzed via One-Way ANOVA unless otherwise indicated with Tukey post hoc test and $p<0.05$ defined as statistically significant. Data is presented as mean ± standard deviation (SD) or mean ± standard error of the mean (SEM) unless otherwise noted.

RESULTS AND DISCUSSION

Similar performance of a novel, rigid cryogenic container compared to cryobags and cryovials

The CryoCase container represents a novel solution for the cryopreservation and storage of cell therapies like CAR-T cells. Unlike cryobags, the CryoCase has a solid wall construction (Figure 1A) to improve rigidity and integrity. Like cryobags, it can hold a flexible volume (15–75 mL) and has multiple attachment points for bioprocessing and

spike ports for infusion and administration at clinical sites (Figure 1A). Given the different thickness and construction of the CryoCase, we sought to test how it would perform and preserve cell functionality during a controlled rate freezing process originally developed for cryobags. T cells from three independent donors were activated and expanded in G-Rex flasks and transduced with a third-generation CD19 CAR lentivirus to generate CAR-T cells. After enough CAR-T cells were expanded, the cells were harvested, washed and formulated before being aliquoted into the different cryogenic containers for cryopreservation. Two different CRFs—one that uses liquid nitrogen for rapid cooling, and another that is liquid nitrogen-free and more compact—were used to cryopreserve CAR-T cells filled into CryoCases, cryobags, or cryovials. CryoCases and cryobags were filled with CAR-T cells at the same viable cell density, 15×10^6 cells/mL, and with the same volume of formulated CAR-T product, 20 mL. Given their smaller size, cryovials were filled with 1 mL of the same CAR-T product. All containers were filled and cryopreserved in CRFs concurrently using previously published protocols [6]. After freezing, all containers were immediately transferred to liquid nitrogen freezers for longer term storage before thawing and cell analysis.

Both cell viability and cell density were remarkably similar across the different cryogenic containers. Using either CRF, the cell viability was above 85% post-thaw for CAR-T cells frozen in CryoCases, cryobags, or cryovials (Figure 1B). To simulate a delayed administration of the CAR-T drug product, cells in each container were held for 3.5–4 h at room temperature to compare changes in cell viability in each of the different containers. Cell viability was remarkably stable in each cryogenic container and remained above 85% even after 3.5–4 h at room temperature (Figure 1B). Cell density was likewise similar across all cryogenic containers (Figure 1C). Cell recovery—defined as the proportion of viable cells recovered post-thaw compared to the calculated number of viable cells originally filled into each container—was 85–90% for CryoCases, cryobags, and cryovials (Figure 1D).

These results suggest that controlled rate freezing programs developed for cryobags and cryovials can be used with comparable performance with the rigid-walled CryoCase. This is supported by the consistent results across two different types of CRFs, each using a different freezing program previously developed for cryobags.

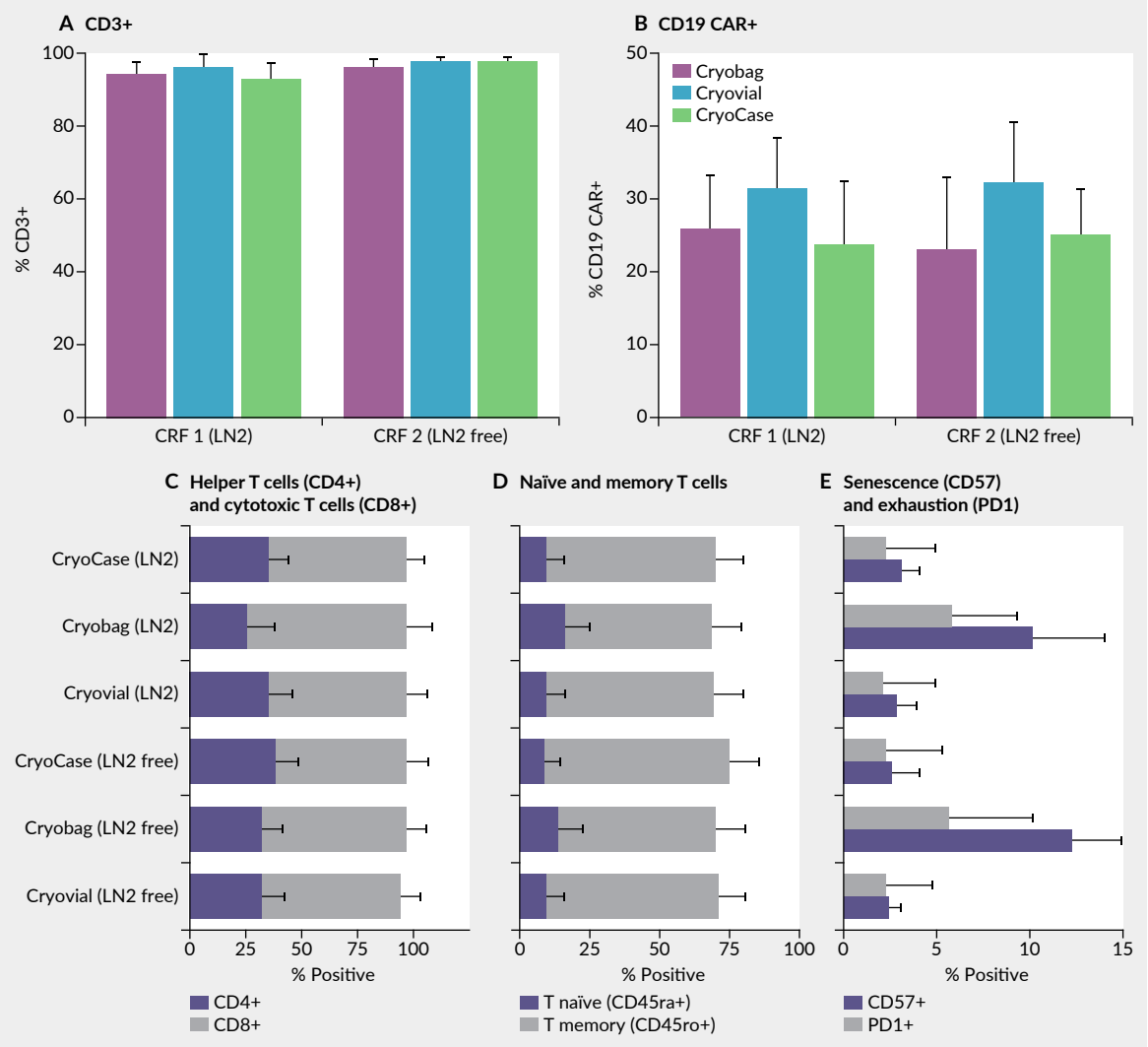
Consistent phenotype and functionality of CAR-T cells across different cryogenic containers

To test the consistency of critical quality attributes of the CAR-T product, we performed a controlled thaw on the different cryogenic containers and compared their phenotypic composition and expression of functional cell surface markers. Cellular identity was consistent across all the cryogenic containers tested. Over 95% of cells expressed the pan T cell marker CD3 (Figure 2A). The proportion of CAR-positive T cells in the thawed product was also comparable between the different cryogenic containers, regardless of the CRF used for cryopreservation (Figure 2B), with 25–30% of CD3 T cells expressing the CD19 CAR averaged across the three different donors. The proportion of CD4+ helper T cells to CD8+ cytotoxic T cells was similar in CryoCases, cryobags, and cryovials (Figure 2C).

T cell phenotype was consistent across the different cryogenic containers. There was a similar proportion of CD45ro+ memory T cells (T-memory) and CD45ra+ naïve T cells (T-naïve) in CryoCases compared to cryobags and cryovials (Figure 2D). This phenotypic consistency across the different containers suggests that cryopreservation and storage in CryoCases does not significantly alter the CAR-T cellular product compared to freezing and storage in cryobags or cryovials.

FIGURE 2

CAR-T cell phenotype and functionality across different cryogenic containers.



(A) Expression of the T cell marker CD3 for the different cryogenic containers. (B) Proportion of CD3+ CD19 CAR+ T cells for each cryogenic container. (C) Ratio of CD4+ helper T cells to CD8+ cytotoxic T cells and (D) ratio of CD45ra+ naïve T cells to CD45ro+ memory T cells from each cryogenic container. (E) Comparison of the senescence marker CD57 and the exhaustion marker PD1 on T cells from each cryogenic container. Data shown: mean ± SEM; n = 3 donors. Analysis performed on cells from each container immediately post-thaw.

To gauge the functionality of CAR-T cells cryopreserved and stored in the different containers we looked at expression of the T cell exhaustion marker PD1 and the T cell senescence marker CD57. Expression of these markers was very low across all the cryogenic containers, with less than 5% of T cells expressing PD1 and less than 15% of T cells expressing CD57 (Figure 2E). Interestingly, cells cryopreserved in cryobags showed a slight increase in expression of these exhaustion and senescence markers (Figure 2E), but this difference was not statistically significant across the three donors tested ($p>0.05$, One-Way ANOVA). This data suggests that the functionality of the CAR-T cells is likewise preserved during cryopreservation in the CryoCase relative to freezing in cryobags and cryovials. However, future studies will need to look at metrics of

CAR-T potency, such as cytokine secretion or target tumor cell killing, to better assess if the functionality of CAR-T cells is similar across the different cryogenic containers.

Scaling product volume in the CryoCase container

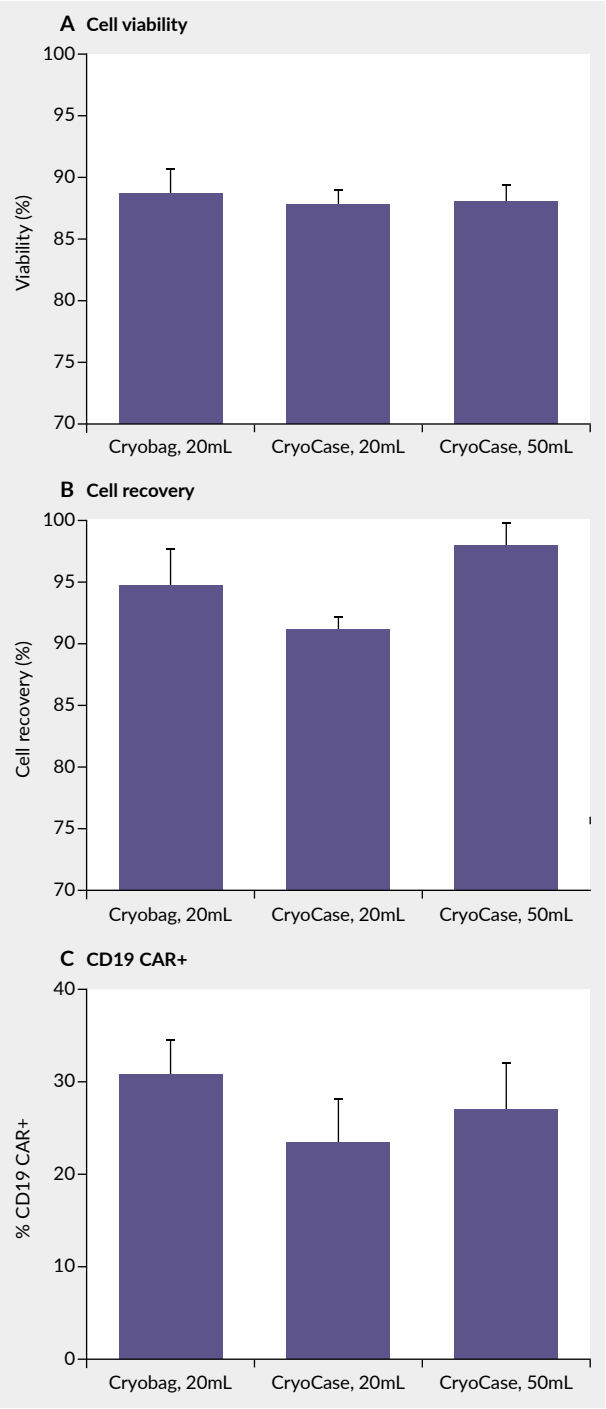
To test how cryopreservation in the new CryoCase container might be affected by the cell product volume, we also filled CryoCases with 50 mL of the same CAR-T product used for the 20 mL fill study of the cryobags and CryoCases. These higher-volume CryoCases were frozen using a liquid nitrogen CRF alongside the CryoCases and cryobags filled with 20 mL of product (due to capacity restraints in the liquid nitrogen-free CRF, additional higher volume samples could not be run). We found that cell viability was again high, above 85% for CryoCases filled with 50 mL and hence similar to the 20 mL fill conditions (Figure 3A). Cell recovery was above 95% for CryoCases filled with 50 mL of CAR-T product (Figure 3B), and CD19 CAR expression was similar to 20 mL cryobags and 20 mL CryoCases at 25–30% (Figure 3C). Cellular identity and phenotype were likewise uniform for the CryoCases filled with 50 mL of CAR-T cells compared to the CryoCases and cryobags filled with 20 mL of CAR-T cells (data not shown). These results suggest comparable performance for controlled rate freezing of CryoCases at different fill volumes, with no change needed in freezing profiles for CryoCases, even at larger product volumes.

Suitability of CryoCase container in an automated and closed CAR-T manufacturing process

We next sought to perform a case study to evaluate how the CryoCase container performs in an automated, full-scale CAR-T manufacturing process. We used the CliniMACS Prodigy system from Miltenyi Biotec to isolate T cells and activate and transduce them according to the manufacturer's recommended protocols [8]. After 8 days

FIGURE 3

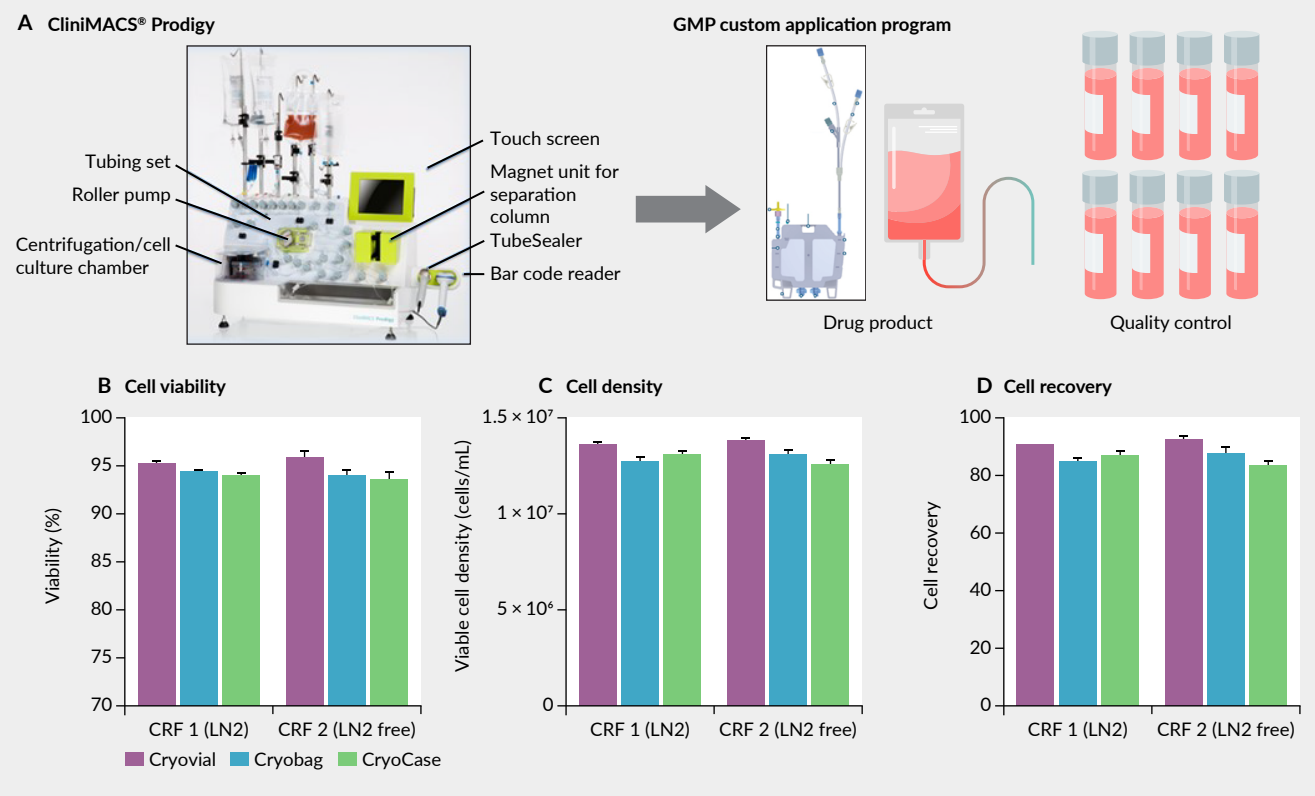
Scaling CAR-T product volume in the CryoCase container.



(A) Cell viability, (B) cell recovery, and (C) ratio of CD19 CAR+ T cells in CryoCases filled and frozen with 50 mL of formulated CAR-T product compared to CryoCases and cryobags filled and frozen with 20 mL of the same CAR-T product. Data shown: mean ± SEM; n = 3 donors.

FIGURE 4

An automated and closed CAR-T manufacturing process utilizing different cryogenic containers.



(A) Overview of the CliniMACS Prodigy instrument for the fully automated and closed production of CAR-T cells, with formulation and fill-finish of the CAR-T product into different cryogenic containers via a GMP compliant CAP developed for the Prodigy. (B) Cell viability, (C) cell density and (D) cell recovery of CAR-T cells manufactured on the Prodigy after cryopreservation in each type of container. Data shown = mean ± SD, n = 1 donor. Picture of CliniMACS Prodigy from Miltenyi Biotec (miltenyibiotec.com). CAP: custom application program.

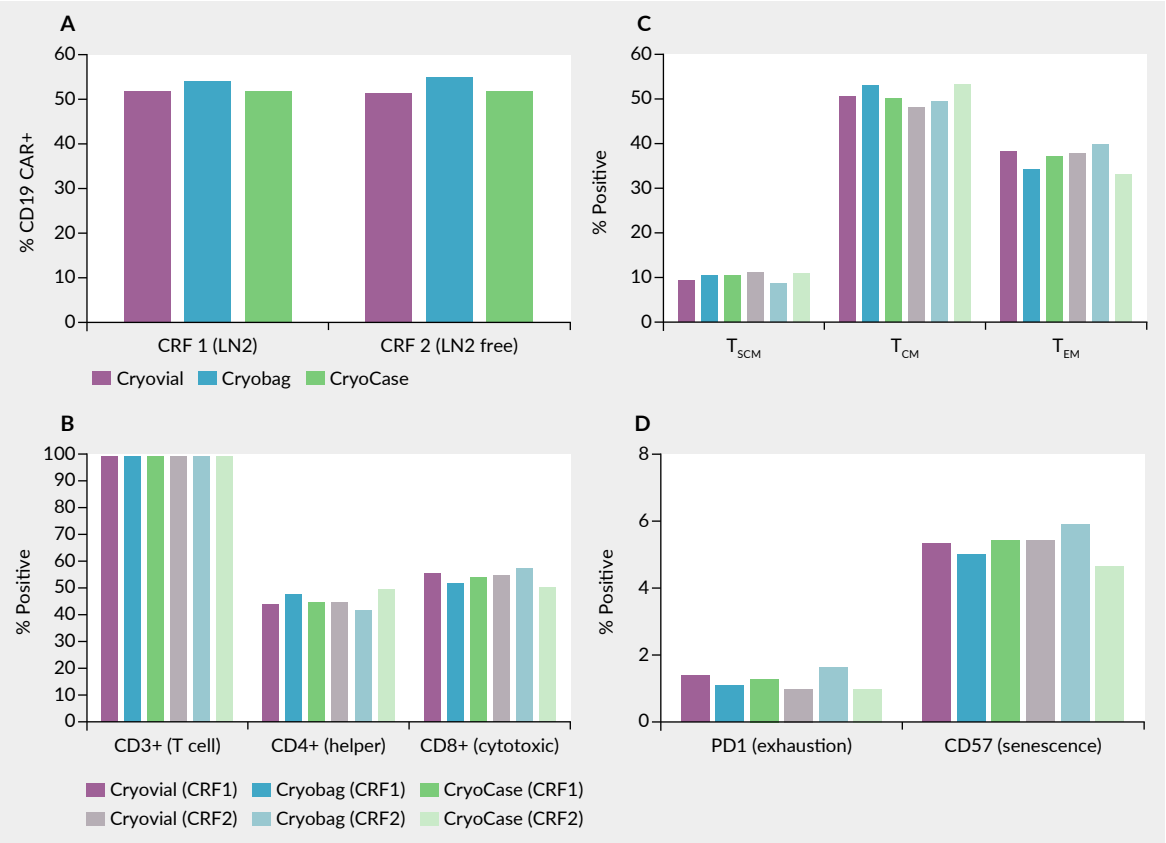
of culture, the Prodigy was able to wash, formulate, and transfer the final CAR-T product into both cryobags and CryoCases via a GMP-compliant custom application program (CAP) developed for fill-finish of the Prodigy (Figure 4A). This CAP allows for a fully automated and closed CAR-T manufacturing process, with both cryobags and CryoCases attached to the Prodigy via sterile welding, and quality control vials further sub-aliquoted from a sample pouch attached to the Prodigy. Unlike cryobags, the CryoCase container did not require air removal from the container after fill-finish, thereby eliminating a step in the manufacturing process.

The CryoCase container delivered a CAR-T product with key critical quality attributes comparable to both cryobags and cryovials. Post-thaw cell viability across all cryogenic containers was above 90% (Figure 4B), and no appreciable difference was observed in cell density of the final product for the different containers (Figure 4C). Cell recovery was similar and above 80% for cryovials, cryobags, and CryoCases (Figure 4D), with no difference observed for cryopreservation in either liquid nitrogen or liquid nitrogen-free CRF.

Cell phenotype was also consistent across the different cryogenic containers and CRFs. CD19 CAR expression was between 50–55% for each of the cryogenic containers (Figure 5A). Over 95% of cells expressed the pan T cell marker CD3 (Figure 5B), and the proportion of CD4 helper T cells

FIGURE 5

Critical quality attributes of a manufactured CAR-T cell product cryopreserved in different containers.



(A) Proportion of CD19 CAR+ T cells from different cryogenic containers for a cell therapy product manufactured, formulated and filled on the CliniMACS Prodigy. (B) Ratio of CD3+ T cells, CD4+ helper T cells, and CD8+ cytotoxic T cells after cryopreservation, LN2 storage, and controlled thawing in each type of cryogenic container. (C) Phenotypic breakdown of CAR+ T cells across different cryogenic containers after thawing. TEM: effector memory T cells, CD45RO+, CCR7-; TCM: central memory T-cells, CD45RO+, CCR7+; TSCM: stem cell memory T-cells, CD45RA+, CCR7+ (D) Portion of PD1+ T cells and CD57+ T cells for different cryogenic containers. Data shown: mean +/- SD; n = 1 donor. SD: standard deviation.

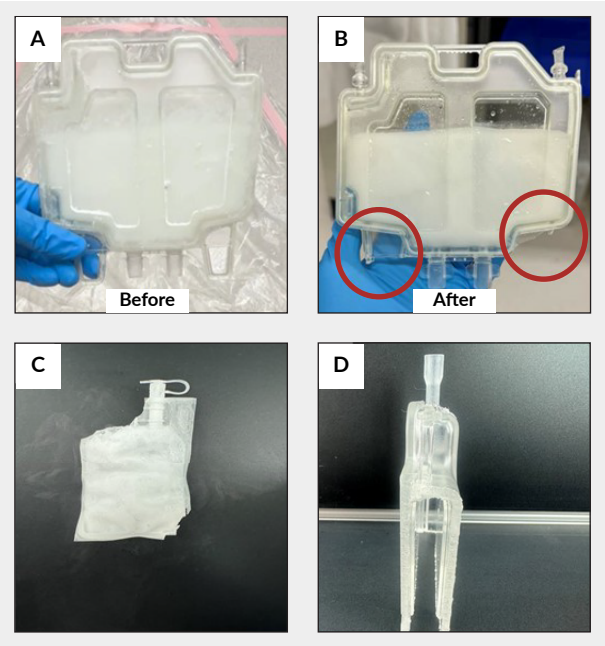
to CD8 cytotoxic T cells was again similar for each of the different containers. In all containers, most T cells were a central memory (CD45RO+ CCR7+) or effector memory phenotype (CD45RO+ CCR7-), with less than 10% comprising a stem cell memory phenotype (Figure 5C). This was uniform across the different cryogenic containers and CRFs tested. Expression of the exhaustion marker PD1 and the senescence marker CD57 was low for all cryogenic containers tested, with less than 7% of T cells expressing either of these markers in the final product (Figure 5D). These results underscore the phenotypic uniformity and quality of CAR-T cells possible in each type of cryogenic container and highlight how new technologies like the CryoCase can be incorporated into automated and closed CAR-T production technologies and workflows.

Container integrity following drop testing

Ten frozen CryoCase containers and five frozen cryobags were removed from liquid nitrogen and dropped onto a laboratory floor from a height of 2 m. This height was selected to represent the possible impact a frozen

FIGURE 6

Before and after images from the rigidity drop test.



The COC used for CellSeal vials and the CellSeal CryoCase is fracture resistant at -196°C. (A) CryoCase immediately after removal for storage in liquid nitrogen. (B) Ten frozen CryoCases were dropped from approximately 2 m. The only observed failures were at the protective shock absorption legs designed to protect the spike ports. (C) Five cryobags were dropped from approximately 2 m. All five units exhibited catastrophic failures. (D) Cross sectional view of the COC walls and weld bead of the CryoCase. COC: cyclic olefin co-polymer.

container might experience during shipment rather than the potential for drop during transport in the manufacturing or clinical setting. In all cases, the CryoCase was found to have either no damage or only damage to the spike port protectors (legs). All five bags were found to have catastrophic fractures compromising the integrity of the sample-containing area of the bag (Figure 6).

CONCLUSIONS

CAR-T cells cryopreserved in the CryoCase maintain high cell viability and recovery and have a phenotypic profile and functionality on par with those frozen in cryobags or cryovials. Whereas cryobags can be fragile and prone to cracking or breaking, and cryovials can have a limited range for fill volumes and offer less flexibility, the CryoCase may provide a robust yet adaptable system that preserves biological activity and product quality for CAR-T cells. The CryoCase system is compatible in automated and closed CAR-T cell processes and can deliver a cellular product with biology and quality on par with cryobags and cryovials. These results offer insight into a novel cryopreservation process and container for CAR-T cells and show that the new CryoCase is compatible with controlled rate freezing systems and programs already developed for cryobags and cryovials.

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AUTHORSHIP & CONFLICT OF INTEREST

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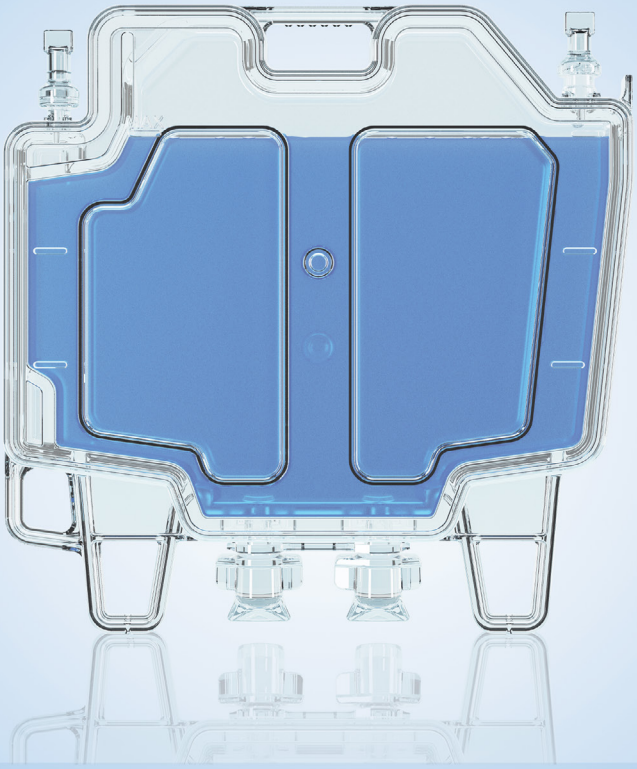

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FASTFACTS

STEMvision™ for CFU analysis of hematopoietic cell therapy products

Colin Hammond, PhD Scientist, Research & Development, STEMCELL Technologies

The development of hematopoietic stem and progenitor cell (HSPC) therapies to cure various blood disorders and genetic diseases requires rigorous quality testing. The colony-forming unit (CFU) assay is a well-established assay to measure HSPC potency, although manual colony scoring is often lengthy and has high variability due to operator subjectivity. This poster explores STEMvision™, an automated imaging and scoring tool that delivers fast, accurate, and reproducible analysis of CFU assays.

ASSESSING THE QUALITY OF HSPC PRODUCTS WITH THE CFU ASSAY

Taking cell and gene therapies from the bench to the clinic requires satisfying regulatory tests for potency, sterility, purity, and identity. The CFU assay is used to determine the frequency of input cells that are able to proliferate and differentiate into erythroid, myeloid, or multipotent colonies in a semi-solid medium, reflecting the potency of the sample. The CFU assay remains the gold standard in vitro potency assay for HSPCs, as it is quantitative and demonstrates a high correlation with clinical transplant outcomes.

REDUCING SUBJECTIVITY IN COLONY COUNTING WITH AN AUTOMATED ANALYSIS

The accuracy of the CFU assay relies on precise scoring of the produced colonies. However, manual scoring methods are inherently subjective and require rigorous training to reduce bias. For example, the variation in counts between trained operators increases with the number of colonies in an assay, indicating that operator subjectivity plays a large role as colonies get more numerous and have a higher frequency of partially overlapping with each other **(Figure 1)**. Automatic scoring instruments like STEMvision™ **(Figure 2)** reduce variation due to their application of validated, consistent algorithms to count and classify each colony. Additional STEMvision™ software add-ons support a 21 CFR Part 11 compliant workflow with system access security policies, audit trails, and electronic signature capabilities.

Figure 2. STEMvision™ instrument for automated CFU assay colony scoring.

Furthermore, the high accuracy of automated counting and classification is illustrated by a high correlation between the colony counts by trained manual operators and the automated counts from STEMvision™ in terms of total colonies **(Figure 3A)**, and distinguishing between the erythroid BFU-E colonies and the myeloid CFU-G/M/GM colonies **(Figures 3B and C)**. External validation has found STEMvision™ to comply with regulatory guidelines for repeatability, inter-operator variability, intermediate precision, and method comparison [1].

FAST AND FLEXIBLE CFU ASSAY ANALYSIS AFTER ACQUISITION WITH STEMvision™

Apart from reducing subjectivity in colony counting, the STEMvision™ instrument also enhances the CFU assay analytical workflow with speed and flexibility. Unlike manual scoring with an inverted microscope, which can be time-consuming, STEMvision™ images a six-culture plate in ~5 minutes with subsequent automated analysis taking less than an additional 10 minutes per plate. Furthermore, the permanent records and CFU assay images enables easy review of results over time, aiding conformity in clinical laboratory settings.

1. Velier M, Chateau A-L, Malenfant C, *et al*. *Cytotherapy* 2019; 21(8): 820–823.

Figure 1. Average number of colonies per CFU assay with coefficient of variation (CV) of replicate counts (%) for manual counting (blue) and automated scoring using STEMvision™ (orange).

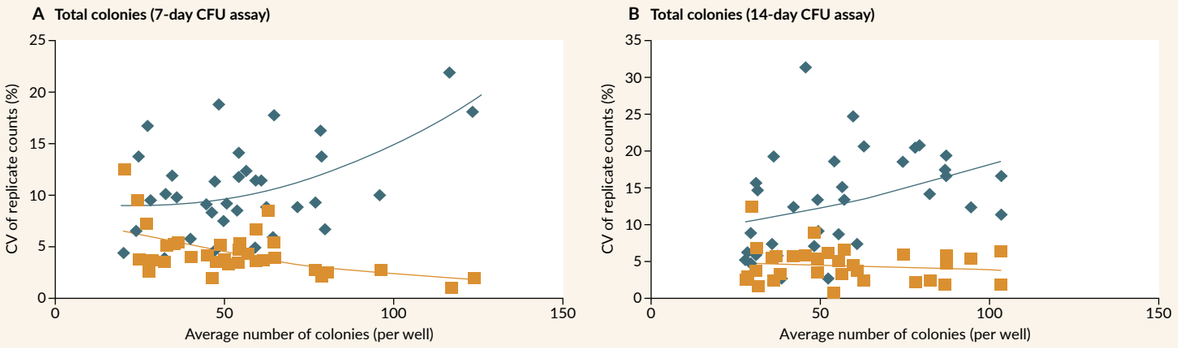
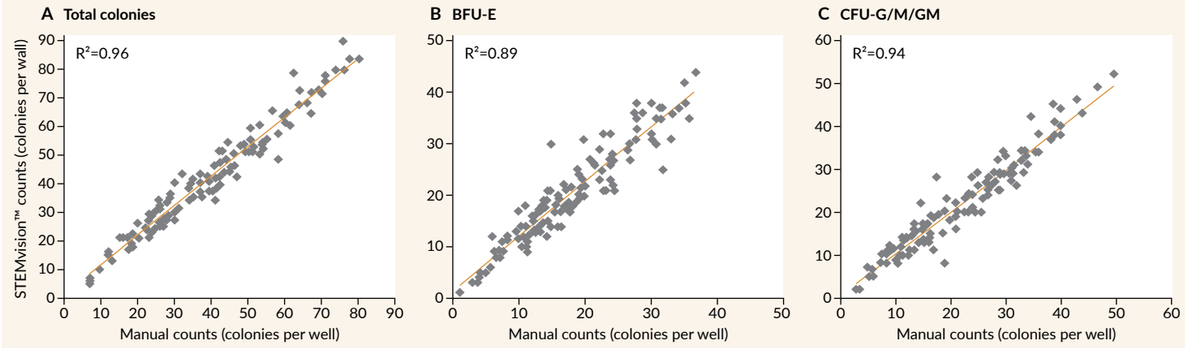


Figure 3. Correlation of automated colony counts with counts by trained operators for CFU assays of previously cryopreserved mobilized peripheral blood cells. (A) total colonies. (B) BFU-E colonies. (C) CFU-G/M/GM colonies.



CELL & GENE THERAPY INSIGHTS

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In partnership with: STEMCELL™ TECHNOLOGIES

AAV capture purification scaling from bench to clinical manufacturing

Kathleen Mihlbachler, Director of Process Engineering, Repligen Corporation

Producing viral vectors at a clinical scale presents significant challenges, including the need to ensure capture and polishing steps are both scalable and reproducible. This poster highlights the critical advancements in chromatography process performance necessary to meet the growing demand for gene therapies, specifically those using AAV vectors.

INTRODUCTION

Traditional viral vector bioprocess technologies often result in low yields and inconsistent product quality. Innovations in chromatography system technology are necessary to ensure robust purification processes that provide consistent, high-quality products. Confirming that capture and polishing processes are reproducible and scalable is also crucial for meeting regulatory requirements, ensuring that final drug products are safe, effective, and consistent across batches.

MEETING DOWNSTREAM PURIFICATION NEEDS WITH THE KRM™ CHROMATOGRAPHY SYSTEMS PLATFORM

Process robustness and reproducibility are key for high productivity and cost-effective viral vector manufacturing at scale. The KRM chromatography systems platform provides state-of-the-art engineering at four commercial scales—the KRM 10 (1–180 L/h), the KRM 20 (6–900 L/h), the KRM 30 (25–2,000 L/h), and the KRM 40 (50–3,600 L/h)—allowing for scale-up of the AAV capture step from the benchtop to GMP manufacturing. The platform features one optimized flow-path design, in which injection molding and overmolded connections at critical junctions eliminate dead legs and decrease hold-up volume. Additionally, the platform utilizes consistent hardware, identical user experience, and OPUS® pre-packed chromatography columns. These features contribute to maintaining quality attributes, improving process recovery, and maintaining process parameters.

Figure 1 displays how this system provides a high level of process control. The KRM 10's gentle fluid management, low hold-up volume, and accurate pump performance can lead to higher recovery and consistency in the scale-up process. This data demonstrates precise and robust gradient performance, which is vital for the polishing step.

CASE STUDY: SCALE-UP OF AAV CAPTURE STEP

A case study was performed to verify the scale-up of an AAV capture step from the benchtop to the manufacturing scale as demonstrated in **Figure 2A** by maintaining quality attributes, such as purity **(Table 1)**, and improving process recovery while maintaining process parameters.

Figure 1. Gradient accuracy of the KRM 10 chromatography system.

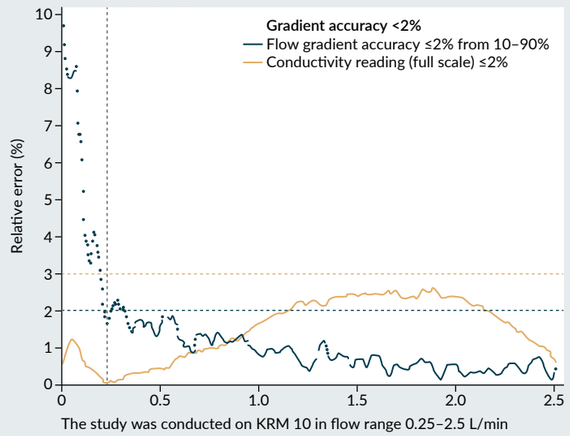
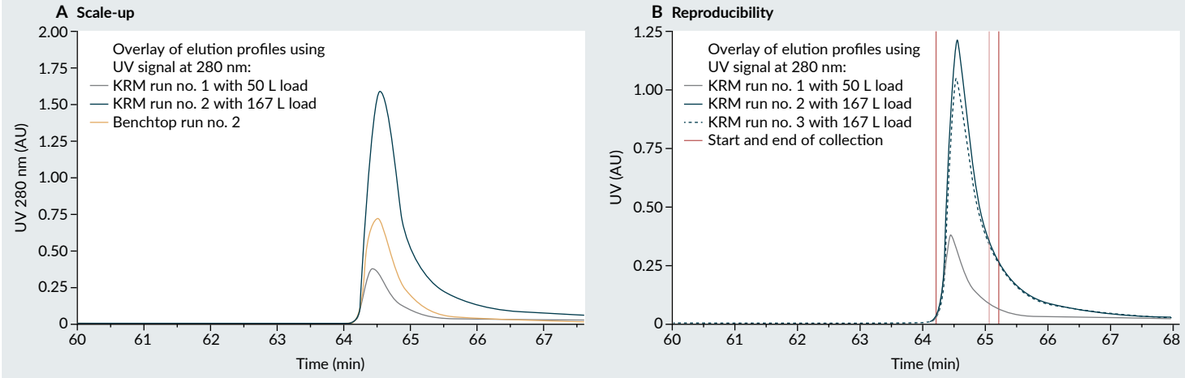


Table 1. Comparison analysis breakdown.

Bench-top				
	Run/sample	Affinity load	Elution	Resin strip
Overall average	Titer (vg/mL)	3.4×10^{10}	2.0×10^{12}	7.8×10^{10}
	Yield (vg)	3.4×10^{13}	1.9×10^{13}	2.0×10^{12}
	Recovery (%)	100	55.8	7.1

KRM™ 10				
	Run/sample	Affinity load	Elution	Resin strip
Overall average	Titer (vg/mL)	3.8×10^{10}	1.2×10^{13}	1.6×10^{11}
	Yield (vg)	5.1×10^{15}	3.2×10^{15}	1.1×10^{15}
	Recovery (%)	100	61.3	10.6

Figure 2. Graphical comparison of AAV capture scale-up study. (A) overlay scale-up; (B) overlay reproducibility.



Furthermore, process robustness and reproducibility of the scale-up results were verified as demonstrated in the overlaid chromatograms in **Figure 2B**, which are key for high productivity and cost-effective viral vector manufacturing at scale.

This study demonstrates that using the KRM 10 chromatography system led to higher recovery and high consistency in the scale-up process through its use of the KRM chromatography system platform.

CELL & GENE THERAPY INSIGHTS

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In partnership with: REPLIGEN INSPIRING ADVANCES IN BIOPROCESSING

FASTFACTS

Accelerate gene therapy downstream process development using scalable pre-packed chromatography columns

Tim Schroeder, Director of Product Management, OPUS Pre-packed Columns, Repligen Corporation

Gene therapies have recently become a key market in bioprocessing, where AAV vectors drive demand for improved, faster development and scaling, and reduced impurities while maintaining yields. The need for both speed to market and enhanced efficacy promotes the utilization of single-use products such as pre-packed chromatography columns. This FastFacts poster will highlight the benefits of miniaturized pre-packed columns and outline how these are applied for the development and scale-up of AAV purification workflows.

PRE-PACKED COLUMNS TO ADVANCE PROCESS DEVELOPMENT

Pre-packed chromatography columns have been successfully utilized in the gene therapy industry over the past 15 years, specifically in the downstream process (DSP) development workflows. These pre-packed chromatography applications include RoboColumns for high-throughput process development, MiniChrom columns for fine-tuning and process optimization on bench-scale chromatography systems, and larger-scale columns for clinical phases and full-scale manufacturing, as illustrated in **Figure 1**. Additionally, pre-packed ValiChrom columns are increasingly being used in process validation where reproducibility and scalable packing performance are crucial.

With the current shift in bioprocessing to focus on new modalities, particularly viral vectors, the pressure to accelerate development timelines for DSP workflows has significantly increased.

Figure 1. Pre-packed chromatography columns for various stages of DSP development workflows.

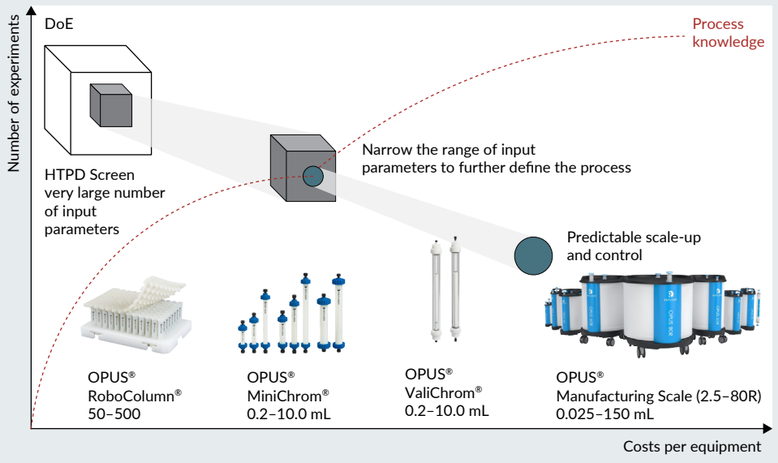
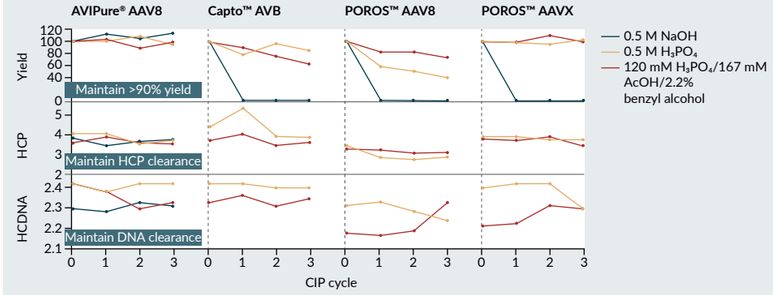


Figure 2. Yield and HCP and hcDNA clearance measured over four cycles across four AAV capture resins evaluated with three different cleaning solutions.



OPTIMIZING DSP WORKFLOWS THROUGH A DoE APPROACH

In a Design of Experiments (DoE) approach to optimize DSP workflows, four different AAV capture resins were evaluated with three different cleaning solutions. Yield and both host cell proteins (HCP) and host cell DNA (hcDNA) clearance were measured over four cycles. In total, 48 fully-automated chromatographic runs were executed in <5 hours. **Figure 2** illustrates that the AVIPure AAV8 resin maintained a consistent yield and HCP and hcDNA clearance for all cleaning solutions and cycles, even when cleaned under caustic conditions. This resulted in a reduction in workflow time when utilizing parallel operations.

The approach was further advanced in a second DoE study where the separation between empty and full capsids was optimized by screening seven different AIEX resins and varying pH for both the wash and elution step with three different salt types. A total of 70 chromatographic runs were executed in <10 hours. These examples demonstrate how screening with RoboColumns contributes to the development of new and robust purification strategies. Calculating the total time savings, both DoE's were completed in less than one day, compared to the typical duration of 45 days.

PRE-PACKED COLUMN PROCESS TRANSFER TO THE BENCH TOP

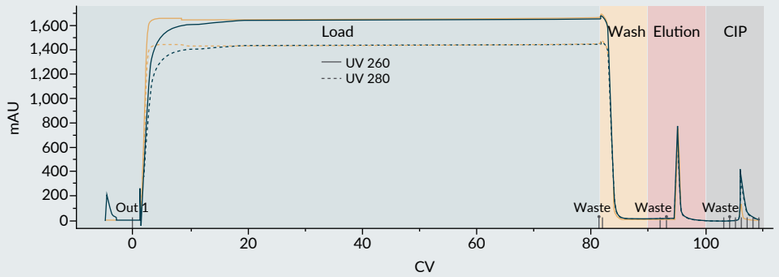
After the successful results of the DoE studies and subsequent data analysis, the scale-up of the purification process was then considered. The data generated with RoboColumns for the AAV capture was translated to the MiniChrom scale. **Figure 3** illustrates a superimposition of four chromatography runs with a 1 mL MiniChrom column pre-packed AVIPure AAV8, operated under optimized conditions.

The superimposition confirms a high reproducibility of results, with consistent yield and hcDNA and HPC clearance.

SUMMARY

AAV vectors drive demand for rapid development and process scaling while minimizing impurities. Pre-packed chromatography columns are crucial to achieving this goal. The DoE approaches optimized AAV purification where both studies were completed in less than a day, compared to the typical duration of 45 days. This demonstrated significant time savings by evaluating capture resins and cleaning solutions and optimizing capsid separation. The data from RoboColumns facilitated a successful scale-up to MiniChrom columns, ensuring reproducibility and efficiency in purification workflows.

Figure 3. Four chromatography runs via 1 mL MiniChrom column pre-packed AVIPure AAV8.



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In partnership with: REPLIGEN INSPIRING ADVANCES IN BIOPROCESSING

Navigating variability and scalability challenges in AAV production

Rafal Garus, Head of MSAT for Clinical and Commercial Production, Viralgen

When manufacturing AAV for gene therapy applications, changes to production processes may be necessary in order to reach the robustness required for commercialization. This poster will outline the key obstacles to assessing comparability after a process change, and solutions for overcoming them.

COMPARABILITY: WHAT AND WHY?

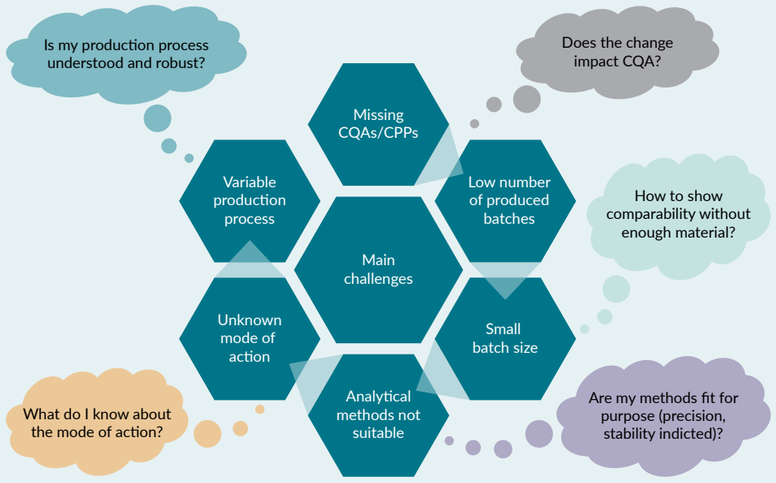
According to the ICH Q5E Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-derived Products Guideline, "the goal of the comparability exercise is to ensure the quality, safety and efficacy of drug product produced by a changed manufacturing process."

Demonstrating comparability does not necessarily mean that the quality attributes of the pre-change and post-change product are identical, but rather that they are highly similar and that the existing knowledge is sufficiently predictive to ensure that any differences in quality attributes have no adverse impact upon the safety or efficacy of the drug product.

KEY CHALLENGES DURING GENE THERAPY COMPARABILITY STUDIES

Important considerations for comparability studies are outlined in **Figure 1**. As gene therapies are commonly developed for rare diseases and therefore produced at a small scale, additional issues can include low numbers of available batches and vials of drug product available for testing.

Figure 1. Main challenges during gene therapy product comparability.

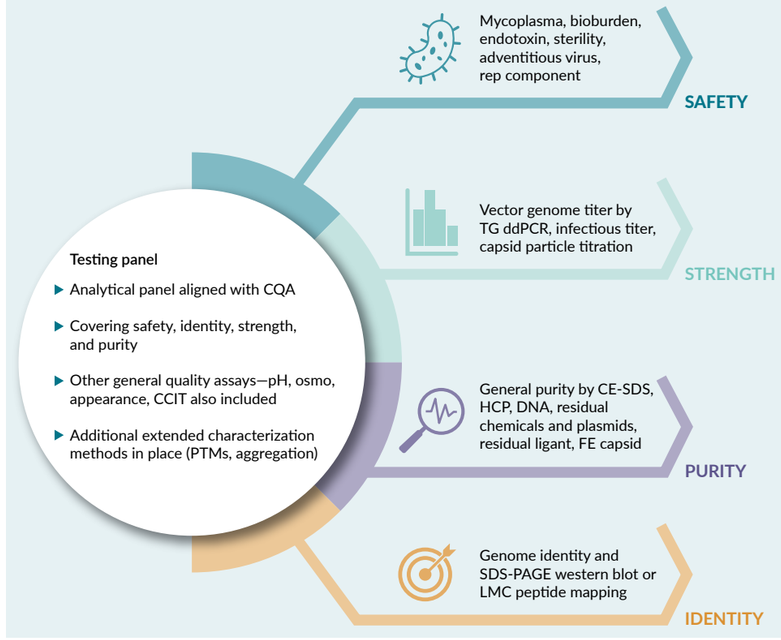


DEFINING ACCEPTANCE CRITERIA WITH HISTORICAL AND PLATFORM DATA

Before making changes to a gene therapy process it is crucial to have well-established and predefined acceptance criteria, in order to enable an objective assessment of whether the pre- and post-change products are comparable.

As a CMDO producing AAV vectors for gene therapy applications, Viralgen has access to a wealth of historical serotype- and product-specific data. A database based on 1,000 produced batches was leveraged to statistically evaluate and calculate acceptance criteras based on platform data, resulting in a strategy designed to ensure successful comparability studies for gene therapies. During comparability, all of the methods required during release testing are used, covering safety, quality and efficacy **(Figure 2)**.

Figure 2. Analytical testing method utilized for release testing and comparability.



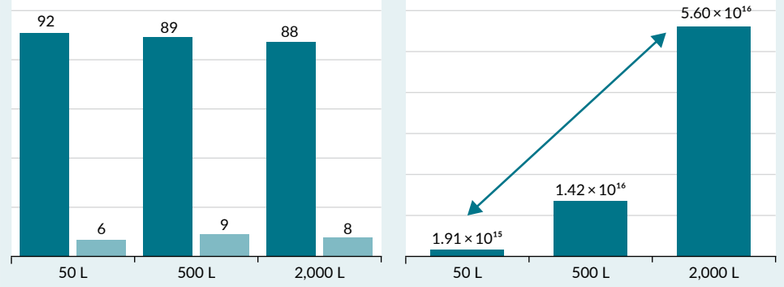
CASE STUDY: SCALING UP AAV PRODUCTION

A comparability study designed to assess linear scalability of the AAV production process is shown in **Figure 3**.

Capsid integrity (full/empty ratio) showed comparable results, and approximately 90% of the produced AAV capsid for all three scales was full. The calculated total viral genome (VG) values confirmed linear scalability between tested scales. Scaling up of the process showed no negative impact on the product quality, safety, and final process productivity.

In summary, implementing platform-validated analytical methods with known and controlled performance, coupled with well understood and characterized production processes and qualified small-scale models, will contribute to success during comparability studies. In turn, such studies ensure that the quality and efficacy of a product—and therefore the safety of patients—is not negatively impacted by process changes.

Figure 3. Linear scaling up of an AAV production process



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