February 2022 Volume 8, Issue 1

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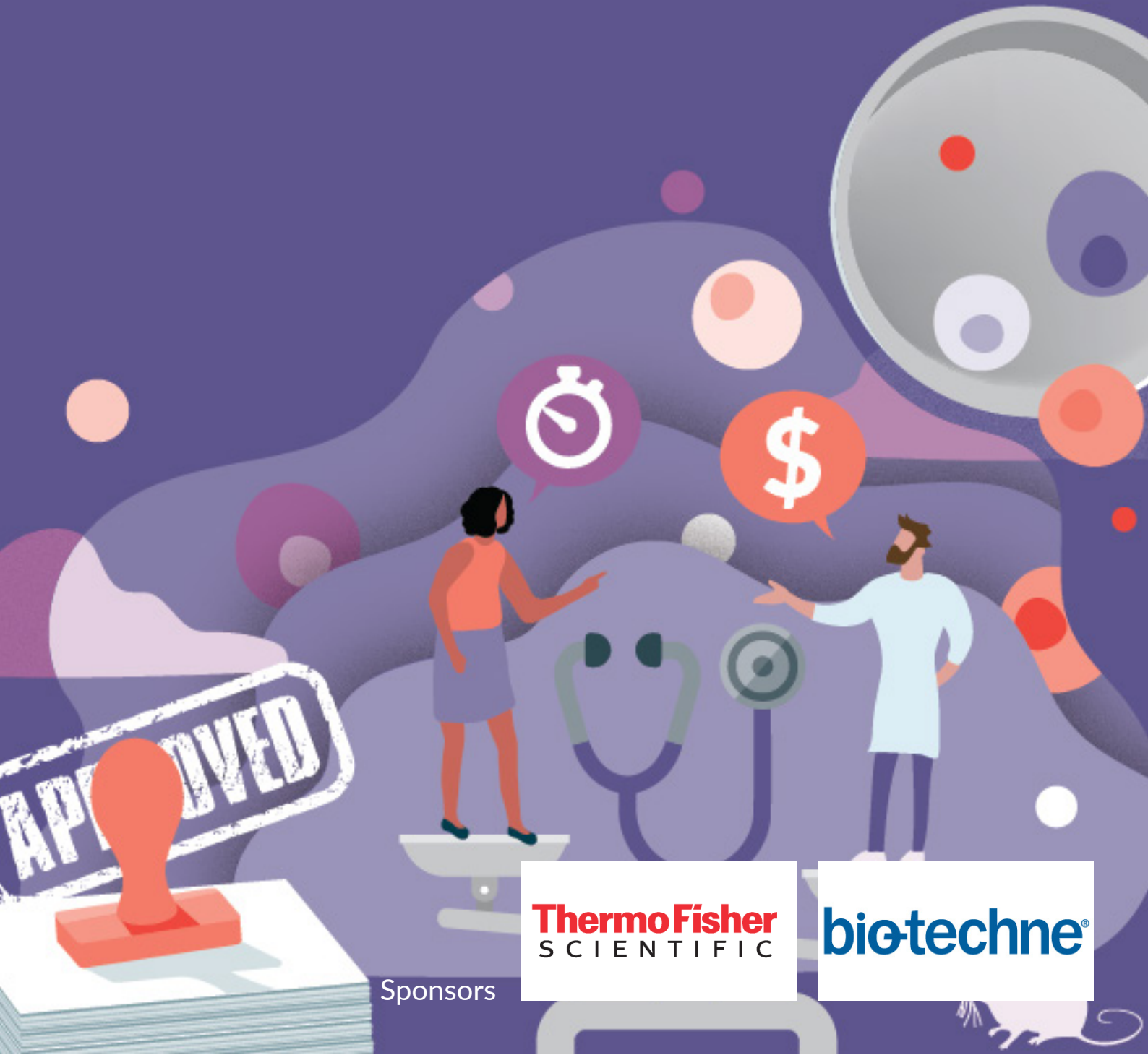

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Considerations in establishing meaningful clinical endpoints in Duchenne muscular dystrophy & other neuromuscular disorders

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Advancements in cell and gene therapy and other cutting-edge technologies are enabling the development of novel therapies for diseases that have significant unmet need. This includes several rare genetic disorders for which there are few or no FDA-approved disease-modifying therapies currently available. Realizing the potential of novel therapeutic approaches in these indications may offer hope to patients but also raises a variety of challenges and questions for drug developers, regulatory agencies, clinicians, payors, and patients themselves. Perhaps the most challenging of these questions is defining what constitutes clinical benefit for patients who are all but guaranteed poor or limited outcomes with currently available interventions and natural disease progression. As cutting-edge therapies advance through clinical development there is an immediate need to answer these questions as well as develop and implement clinical endpoints that are meaningful for all stakeholders.

Cell & Gene Therapy Insights 2022; 8(1), 129–140

DOI: 10.18609/cgti.2022.003

The evolving landscape of clinical endpoints to support the development, approval, and commercialization of gene therapies for the treatment of Duchenne muscular dystrophy (DMD) provides an excellent example in which to consider the limitations of classical endpoints and the potential of new strategies being explored. Several novel endpoints currently under evaluation, including technology-based assessments

[1–3] and patient-reported outcome measures (PROMs), [4] are focused on understanding what functions or activities may be most meaningful to patients, especially at different disease stages, and developing outcomes using the right tools to accurately capture a patient's abilities at a given moment in time. While this article focuses on considerations for selecting clinical endpoints to assess the impact of DMD gene therapy, the examples included here provide a framework for how such outcomes could have utility in other rare neuromuscular disorders for which there is similar significant unmet need.

DMD OVERVIEW

DMD is an X-linked genetic disorder that is the most common form of muscular dystrophy, affecting approximately 1 in 5,000 newborn males [5]. The disease results from mutations in the gene encoding dystrophin, an essential structural protein that stabilizes muscle integrity through its repeated cycles of contraction and relaxation. Over time, muscle that lacks functional dystrophin protein degenerates, resulting in fibro-fatty tissue replacement and a progressive decline in muscle function [6].

Patients with DMD typically achieve developmental milestones until approximately five years of age, at which time disease symptoms usually present as delays in attaining additional motor milestones and associated signs of muscle weakness. Over time, disease progression leads to loss of ambulation and declining cardiopulmonary function, eventually resulting in death primarily due to respiratory or cardiac failure [7,8]. Current life expectancy is in the mid-20s of age, although as a result of earlier interventions survival can extend into later decades of life [9].

While there currently is no cure for DMD, there are a variety of medical interventions used to manage disease symptoms. Physical therapy and mechanical aids for ambulation are commonly used to prolong motor function for as long as possible, while corticosteroids are standard of care therapy for slowing the pace of muscle weakening [6]. Prolonged overall survival in recent years has largely been attributed to improved standard of care, including the more common and earlier use of ventilatory support and cardiac management [1].

Genetic strategies to address the root cause of DMD, the loss of dystrophin, have faced substantial hurdles due to the large size of the dystrophin encoding gene and the requirement for systemic delivery, with dystrophin playing a critical role in muscles throughout the body. Several exon-skipping therapies, which bind to RNA and are designed to enable production of a truncated yet functional dystrophin protein for specific subsets of mutations, have recently received accelerated approval from the U.S. Food and Drug Administration (FDA) [11–14]. To date, these therapies have shown low levels of dystrophin protein restoration but an unclear relationship with functional benefit and, consequently, confirmatory trials have been required to better elucidate the therapeutic impacts of these treatments.

ADENO-ASSOCIATED VIRUS (AAV)-BASED GENE TRANSFER THERAPY AS POTENTIAL TREATMENT MODALITY FOR DMD

As a monogenic disease, gene therapy approaches that enable delivery of DNA sequences that can produce functional dystrophin protein are investigated strategies for the treatment of DMD. Restoration of dystrophin activity is expected to correct the underlying cause of DMD, preserving and potentially improving muscle function and extending survival. While multiple viral vectors have been evaluated for therapeutic delivery of the dystrophin gene, concerns related to immunogenicity and genomic integration have dissuaded the use of approaches involving adenoviruses or lentiviruses, establishing AAV-based approaches as the preferred

DOI: 10.18609/cgti.2022.003

delivery mechanism for systemic gene transfer. However, the coding sequence of the dystrophin gene, which is approximately 11 kilobases (kb) in length, exceeds the limited 4.7 kb packaging capacity of AAV-based vectors [15,16]. As a result of these limitations, shortened versions of the dystrophin gene (termed microdystrophins) have been developed to retain important functional elements of the full-length protein and fit within the AAV vector capacity. The potential for microdystrophin to provide benefit to patients with DMD is in part based on the observation that patients with Becker muscular dystrophy (BMD), a milder dystrophinopathy, typically have less severe symptoms despite mutations in the dystrophin gene, as they still produce a truncated but partially functional dystrophin protein [16]. While a microdystrophin gene therapy would differ from BMD in that expression of the therapeutic transgene would occur after postnatal administration, the progressive nature of the disease suggests that intervention at different stages would still have the potential to slow, stabilize, or improve a patient's overall disease trajectory depending on their baseline function at the point of treatment.

Extensive research into understanding the unique and critical functionality of components of the full-length dystrophin protein has resulted in the development of recombinant microdystrophin expression constructs that include critical domains such as the actin and dystroglycan binding domains, hinge regions and spectrin-like repeats [17,18]. Additional work has also uncovered the role of the R16/R17 spectrin-like repeats in localizing neuronal nitric oxide synthase (nNOS) to the muscle fiber sarcolemma (Figure 1), a feature that has been identified as being critical for preventing ischemia-induced muscle damage [19–21]. Solid Biosciences is currently evaluating SGT-001, an AAV-based microdystrophin gene therapy that includes the R16/R17 nNOS binding domain, for the treatment of DMD in the ongoing IGNITE DMD Phase I/II clinical trial (NCT03368742). SGT-001 was developed to drive the systemic skeletal and cardiac muscle expression of a uniquely functional microdystrophin using the muscle specific promoter CK8 and the muscle-tropic capsid AAV9.

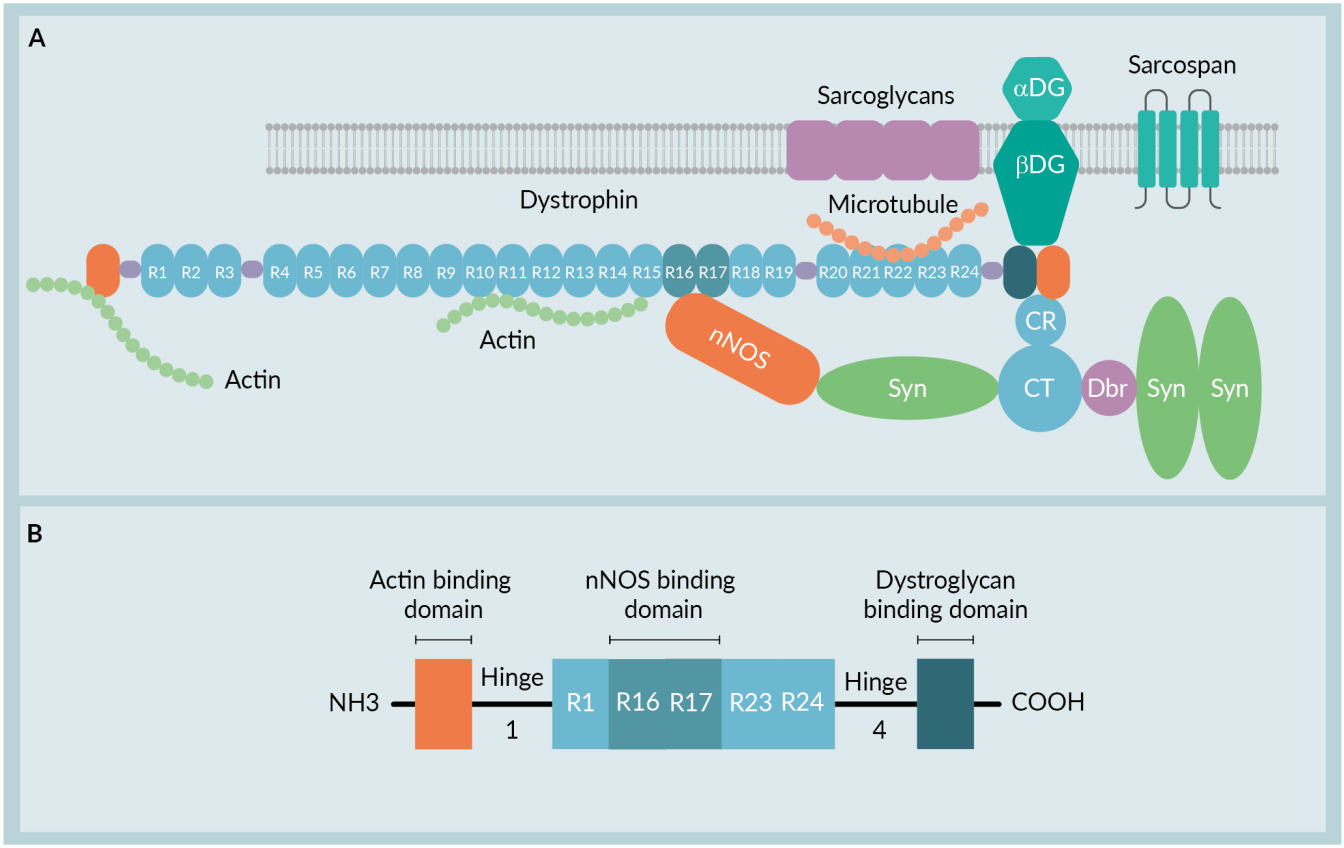
AAV vectors, which in nature are non-pathogenic to humans, offer an efficient approach to deliver potentially therapeutic DNA constructs to target tissues for a variety of disease indications. AAV-based therapies have demonstrated safety and efficacy in clinical trials, leading to regulatory approvals in both the United States and Europe of AAV2-based LUXTURNA, [22,23] for the treatment of confirmed biallelic RPE65 mutation-associated retinal dystrophy and AAV9-based ZOLGENSMA for the treatment of spinal muscular atrophy (SMA) [24,25]. Benefits of AAV vectors include the ability to achieve systemic delivery to target tissues via intravenous administration and tissue-specific transgene expression through the use of targeted promoters [26], especially critical for diseases such as DMD, and large-scale manufacturability for commercial use. However, as an evolving platform for therapeutic development there are still several challenges to using AAV vectors for DMD gene therapy, including the requirement for high viral doses to reach target tissues throughout the body and managing vector-related immune responses. In addition, strategies aimed at dosing patients who have pre-existing neutralizing antibodies to AAV and approaches for repeat dosing of AAV-based therapies are important ongoing fields of research.

DEFINING CLINICAL ENDPOINTS IN TRIALS OF INVESTIGATIONAL DMD GENE THERAPIES

In addition to the gene therapy-specific challenges related to vector engineering, manufacturing, and dosing, another important hurdle in developing novel therapies for DMD is defining clinical trial endpoints that are meaningful to physicians, patients, their caregivers, and regulatory agencies. An important first step in achieving this goal is acknowledging

FIGURE 1

Dystrophin and SGT-001 Microdystrophin Functional Domains.



A: Dystrophin and the glycoprotein complex (Modified from McGreevy JW *et al. Disease Models and Mechanisms*, 2015) [12]. B: SGT-001 Microdystrophin containing the neuronal nitric oxide synthase (nNOS) binding domain.

the limitations of classical endpoints used to date in DMD trials.

The 6-Minute Walk Test (6MWT), North Star Ambulatory Assessment (NSAA), and Performance of Upper Limb (PUL) are tools commonly used to evaluate aspects of DMD patients' motor function. Despite their utility, all three have limitations.

- The 6MWT was not designed specifically for patients with DMD and therefore may carry a potentially significant motivational bias in the younger patient population able to perform the test. Although younger DMD patients are clearly ambulatory, they may not fully understand the request or be willing to complete the relatively long-duration of the assessment, which introduces the potential for inconsistency [27].
- The NSAA was developed for DMD and can provide important information on changes in a patient's ability to perform specific tasks associated with ambulation. However, the test's scoring system may lack the sensitivity to accurately distinguish between one's levels of functionality across each item. In addition, substantial scorer training is required to limit inter-observer variability, and overall, the test may not capture the most relevant aspects of daily life for all DMD patients [28,29].
- PUL was developed to assess function of late-stage ambulatory and non-ambulatory DMD patients but lacks the sensitivity to capture changes in younger patients and overall can miss what matters most in a patient's daily life and for their overall health [30].

DOI: 10.18609/cgti.2022.003

Given the inherent variability in these more classical endpoints, the methods used to evaluate motor function and capture meaningful change in a patient's disease course must continue to constantly evolve [31]. In general, DMD patients tend to prioritize physical functions that are essential for activities of daily living, such as participating in school and social activities, getting in and out of a car, and eating without assistance, over changes measured by these clinical assessments. Performance in all of these tests can be dependent on a patient's energy level, degree of motivation and mood at the time thereby increasing variability and making interpretation difficult. The development of newer tools to measure important functional changes more objectively, such as activity monitors and wearable sensors, may provide important insight into where a patient is on their individual disease trajectory.

DEFINING MEANINGFUL ENDPOINTS IN DMD GENE THERAPY CLINICAL TRIALS

Protein biomarkers

As a nascent field, correlations between microdystrophin expression levels and other functional endpoints in gene therapy clinical trials are not yet fully established. However, an analysis of dystrophin quantities in dystrophinopathies has shown that even low levels of dystrophin correlate with substantially different outcomes, supporting the meaningfulness of quantifying expression [32]. Similarly, a study in patients with other dystrophin mutations (X-linked dilated cardiomyopathy) [33] and studies in animal models of DMD demonstrate that even below-normal levels of dystrophin provide substantial functional benefit [34,35]. Increased dystrophin expression to levels of <1–5.9% of normal have been used as the basis for the FDA accepting accelerated approvals of exon-skipping therapies for the treatment of DMD in patients with specific exon mutations [11–14].

Similarly, ongoing DMD gene therapy trials collect muscle biopsies to assess the quantity of expressed microdystrophin using techniques such as Western blot and mass spectrometry. In addition to measuring protein levels, supportive techniques such as immunofluorescence are used to characterize the molecular functionality of the protein. Evaluation of microdystrophin localization and its ability to recruit and stabilize members of the dystrophin associated protein complex, such as β-sarcoglycan and nNOS, demonstrate key processes essential for restoring muscle membrane integrity in the absence of dystrophin. Long-term evaluation of the clinical benefit of microdystrophin gene therapies may guide whether measurement of microdystrophin expression can act as a surrogate marker of efficacy.

Additional approaches are also underway to further evaluate circulating proteins to better understand whether minimally invasive blood or even urine-based measurements may provide additive insight into a patient's disease course. However, for established markers that are evaluated clinically for the diagnosis of DMD, such as serum creatine kinase (CK), variability in absolute levels and decreases associated with loss of muscle mass rather than improving condition have made interpretation of changes difficult. To date, reliable circulating biomarkers indicative of prognostic differences or varying stages of disease have not yet been identified.

Technology-based assessments of muscle function

Wearables and video-based assessments capture real-world changes in motor function

As discussed above, there is a lack of sensitivity and objectivity in many of the standard clinical assessment tools used as DMD endpoints. The increasing availability of digital

and wearable technologies, as well as artificial intelligence and machine learning programs that are capable of complex data analytics, is enabling new approaches to assess muscle function in the clinic and, importantly, in real time and in real-world settings. In 2019, the European Medicines Agency approved stride velocity rate 95th centile (SV95C) as assessed with ActiMyo as an endpoint for ambulatory DMD patients [1]. ActiMyo is a wearable device that uses magneto-inertial technology and advanced algorithms to capture and analyze position, orientation, navigation, and motion data with high precision as the patient moves through his daily activities. The device provides a 3-dimensional characterization of the patient's motor function in real time and can quantify changes in this function over time [1]. In a non-biased approach, artificial intelligence is being used to analyze kinetic data captured from sensors either worn at the wrists and/or ankles or embedded in a wearable body suit to develop an objective, digital biomarker of DMD disease progression based on changes in movement patterns over time. Interim data from a trial evaluating this approach, the KineDMD study, has already identified motor coordination differences between patients with DMD and healthy controls [2].

A unique, novel real-world evidence approach to assessing muscle function utilizes videos captured with a smart phone to measure changes in the quality of a patient's movement as they perform tasks at home, with the video then evaluated and scored by blinded, trained raters. Initial results from evaluations of this Duchenne Video Assessment (DVA) [3] tool demonstrate its ability to differentiate among severity groups based on movement quality.

These types of approaches will allow assessment of function to be based on patients' ability to perform day-to-day activities in real-world settings. As such, they may provide novel insights into how DMD patients experience their disease and respond to therapy in a manner that is both objective and more meaningful to patients and their caregivers.

Imaging-based assessments objectively measure changes in muscle composition as a biomarker of function

Quantitative magnetic resonance imaging (qMRI) is an additional tool that is being developed to improve characterization of muscle structure and function by evaluating muscle composition and, importantly, retention of muscle area over time compared with degrees of fatty replacement or overall disease processes. While classical evaluations of active dystrophic pathology and muscle composition require muscles biopsies for pathology analyses, this technique represents a potentially important tool to noninvasively provide greater insight into patients' muscle over longitudinal assessments. A growing body of evidence from natural history datasets is evaluating a potential correlation between MRI results and functional outcomes [36–39] to help inform our understanding of how qMRI and MR biomarkers might be used as endpoints in clinical trials of investigational DMD therapies.

Pulmonary function

Pulmonary failure is a predominant cause of mortality in DMD, prompting assessments of pulmonary function to routinely be performed in patients [8]. However, despite the characterization of progressive decline in pulmonary function over time, pulmonary function tests (PFTs) have not routinely been used as outcomes in therapeutic development for DMD. As early prevention of functional declines may lead to greater maintenance of functional capacity, [40] PFTs may represent meaningful therapeutic endpoints to evaluate in clinical trials.

Pulmonary function is measured directly using standard PFTs such as forced vital capacity (FVC % predicted), peak expiratory flow (PEF % predicted), and forced expiratory volume in one second (FEV1 % predicted). Although these tests may be subject to similar effects of patients' energy, mood, and motivation as the 6MWT, NSAA, and PUL, the methods

DOI: 10.18609/cgti.2022.003

have been established to limit these variables by specifically evaluating maximal responses. In a natural history study evaluating FVC % predicted and PEF % predicted in DMD patients, a consistent annual decline of approximately 5% was observed between the ages of 5 to 24 years, demonstrating the utility of this assessment to characterize disease progression even in younger patients, as well as across the broad spectrum of DMD patients regardless of their ambulatory status [41]. Importantly, results of this study suggest that modification of the American Thoracic Society acceptability criteria for these PFTs may provide a more reliable approach to assessing pulmonary function over time in DMD patients, given that these patients' disease may limit their ability to perform the complete inspiration and exhalation needed to meet the unmodified criteria [41].

PFTs have not routinely been used as endpoints in DMD clinical trials, especially those enrolling younger patients, despite declines being observed as early as 5 years old. Encouragingly, interim data from the IGNITE DMD study of SGT-001, where PFTs were performed, have demonstrated stabilization or improvement in the % predicted FVC, PEF, and FEV1 values in post-treatment assessments compared to baseline, potentially representing meaningful changes to patients' disease courses where declines would otherwise be expected from natural history [42].

Cardiac function

More recent identification of cardiac involvement in younger DMD patients and heart failure as a known cause of mortality in DMD has emphasized the need for cardiac monitoring and management at earlier ages to potentially delay loss of function [43,44]. Natural history studies have characterized the mean onset of cardiomyopathy in DMD as approximately age 16, due to documented declines in ejection fraction and/or fractional shortening [45]. However, subclinical cardiac manifestations preceding systolic functional declines are also frequently identified at earlier ages, prompting preventative intervention [46]. As older, non-ambulatory patients are enrolled in studies, and long-term follow up of younger treated patients occurs, the use of echocardiograms and cardiac MR for the evaluation of changes in systolic function will become increasingly important. Further research into assessments of changes in diastolic function and cardiac fibrosis in younger DMD patients may also uncover additional important outcomes to be evaluated in clinical trial settings.

Measuring activities of daily living

As a disease that ultimately impacts every aspect of patients' daily lives and places great demands on parents and caregivers, laboratory and clinical assessments simply cannot fully capture patients' experience of their disease and the impact of therapeutic intervention. Specific domains of existing patient reported outcome measures (PROMs), such as the Pediatric Outcomes Data Collection Instrument (PODCI), may help to fill the gap in patient experience data from clinical trials of novel DMD interventions. Additionally, new DMD-specific PROMs designed to assess the DMD patient experience also have an important role to play in ensuring that patient perspectives are included in the overall evaluation of the potential benefit profile of investigational DMD therapies [4]. In Solid's IGNITE DMD study, the PODCI is utilized as an important tool to capture the patient experience at moments in time. This more traditional instrument is further supported by the use of a DMD-focused, semi-structured interview-based, qualitative assessment tool designed by Modus Outcomes. Together, interim data from these two instruments indicate that patients treated with SGT-001 show improvements in what may be considered meaningful areas of daily life compared with pre-treatment responses [42].

While these instruments provide information on meaningful aspects of patient's functional capacities, inherent limitations exist due to the nature of being self-reported. Especially in open label trials, patient reported

outcomes have the potential to be impacted by feelings associated with participation and as a result should be coupled with more objective outcomes for better overall interpretability. In addition to these instruments, the digital tools in development to measure motor function are focused on understanding patient perspectives to identify and capture the outcomes that matter most in an objective manner. This emphasis on using real-world assessments in each platform strengthens the ability of these tools to characterize a patient's experience and potentially detect meaningful changes as a result of therapy.

LEVERAGING NATURAL HISTORY AND OTHER DATA SETS TO IMPROVE CLINICAL ENDPOINT DESIGN AND SELECTION

A key challenge in demonstrating benefit in clinical trials of novel DMD therapies is the heterogeneous trajectory of disease. Patients with DMD have a period of early improvement in muscle and pulmonary function before experiencing decline as muscle satellite cells are depleted and repair of damaged muscle slows. Even within this broad trajectory, individual patient experiences can be quite varied, making it difficult to determine whether changes observed in clinical trials are due to therapeutic intervention or the patient's evolving disease status. Improved use of a growing body of natural history data may help to address these challenges, and several collaborative efforts to collect and analyze such data are making progress toward this important goal. These include:

- The Collaborative Trajectory Analysis Project (cTAP), a collaborative organization comprised of academic researchers, statisticians, and industry sponsors that was established specifically to leverage multiple, robust DMD natural history data sets for use in designing more effective clinical trials for investigational DMD therapies. These data are critical for understanding and accounting for variation in DMD disease progression and determining whether changes in endpoints observed in clinical trials result from therapeutic intervention or the natural course of disease. Multiple publications have been generated from this effort that highlight key aspects of DMD disease progression and provide sponsors, physicians, and the community with greater insight into the functional decline that occurs over time [47–50].
- The Cooperative International Neuromuscular Research Group's (CINRG) Duchenne Natural History Study (NCT00468832) is the largest prospective multicenter natural history study to date in DMD, with more than 400 boys and young men with DMD enrolled since 2006, with the data accessible by sponsors and others for uses such as clinical trial design.
- The Critical Path Institute (C-Path), which is utilizing multiple available datasets, is a public-private partnership focused on catalyzing novel approaches to medical and regulatory science. It opened its Duchenne Regulatory Science Consortium (D-RSC) database to qualified researchers through its Rare Disease Cures Accelerator, Data and Analytics Platform (RDCA-DAP®) in April 2021, providing access to a centralized and standardized infrastructure for DMD-related data, with the goal of accelerating the development of novel DMD therapies and enhancing the understanding of the natural history of DMD.

COLLABORATION IS ESSENTIAL ACHIEVING CONSENSUS AND ADDRESSING STAKEHOLDERS' NEEDS

With multiple stakeholders bringing unique needs to this discussion, defining clinical and regulatory endpoints for DMD gene therapy must be a collaborative effort that includes and reflects the needs and priorities of patients, physicians, industry, regulators, and payors. The DMD community has taken an

DOI: 10.18609/cgti.2022.003

active approach to collaboration with several independent efforts initiated to advance understanding of the disease and improve the chance of regulatory success. Ongoing collaborative efforts in this area include:

- The Pathway Development Consortium (PDC), which was launched in 2021 as a public-private multistakeholder initiative focused on identifying, developing, expanding, and maintaining pathways to effective therapies for patients diagnosed early in life with rare diseases. The PDC seeks to achieve these goals by bringing together a broad and diverse group of stakeholders from the rare disease and AAV gene therapy communities, including patients, industry, regulators, academia, and payers, for meaningful scientific and policy discussions. In November 2021, the PDC released a draft white paper proposing a framework leveraging the FDA's Accelerated Approval pathway to address the urgent unmet need for diseases that may be treated with AAV gene therapy, including DMD, which is the PDC's initial focus indication [51].
- The Bespoke Gene Therapy Consortium (BGTC), a public-private partnership being led by the FDA and NIH, was established to create tools for streamlining the gene therapy development process with the goal of reducing development costs and encouraging biopharmaceutical companies to innovate gene therapies for rare genetic diseases, including DMD.

Given the expanding portfolio of investigational DMD therapies, including gene therapy, these organizations have important roles to play in establishing a consensus on meaningful DMD clinical trial endpoints in a timely manner. As innovative and potentially transformative therapies, gene therapies and other breakthrough technologies offer tremendous opportunities to improve the lives of patients with DMD. As a result, it becomes increasingly important to be able to design appropriate, sensitive clinical studies to test the effectiveness of these experimental drugs. Realizing those opportunities demands that all stakeholders work together to establish agreed-upon outcomes that fully reflect the heterogenous nature and lifelong impact of DMD on patients and their caregivers. Therapeutic innovation should strive to define novel approaches for assessing therapeutic impact for the many individuals whose lives are impacted by DMD.

CONCLUSION

Current DMD clinical trial endpoints have limitations in their ability to accurately assess meaningful changes in a patient's disease course. Consequently, developing novel DMD therapies may also require evolution of current regulatory frameworks to consider novel surrogate and supportive endpoints that are clinically meaningful to the patients and could be useful for accelerated approval pathways. This evolution is already in process, with the growing acceptance of real-world evidence in regulatory decision making and increased willingness to consider and approve new surrogate endpoints. Early and collaborative dialog among key industry, regulatory, clinical, scientific, and patient stakeholder groups will be essential for assessing feasibility and validating new endpoints for accelerated approval of novel DMD and other neuromuscular disease therapies.

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The authors are all employees of Solid Biosciences and all own stock or stock options in Solid Biosciences. The authors have no other conflicts of interest.*

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

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *Jan 12 2022;* ***Revised manuscript received:*** *Feb 8 2022;* ***Publication date:*** *Feb 18 2022.*

DOI: 10.18609/cgti.2022.003

INTERVIEW

Advancing the application of organoids, tissue-on-a-chip, and other emerging *in vitro* models to support cell and gene therapy

JASON EKERT PhD, MBA, is Senior Director, GSK Fellow and Head of the complex *in vitro* models (CIVM) group at GlaxoSmithKline. He is responsible for an integrated enterprise strategy for R&D applications of complex human-relevant and translatable complex *in vitro* models (e.g. Organoids, Microphysiological systems and bioprinting).
Before joining GSK he worked at Janssen in biotherapeutic drug discovery. Jason received his PhD from Adelaide University. Post-doctoral training was performed at University of California, Davis and Coriell Institute for Medical Research.
He's currently the past chair for the IQ-MPS affiliate. He is a member of the society for lab automation and screening (SLAS) and society of toxicology (SOT).

MARIE DAVIES PhD, is a Senior Director in the Oncology Cell Therapy Research Unit in GSK and has more than 20 years of drug discovery experience in the biotechnology industry and large pharma. Dr Davies has extensive knowledge of drug discovery across biopharms, cell and gene therapies and vaccines with particular interest in the inter-relationships between safety, efficacy and manufacture. Throughout her career she has been accountable for delivering, reviewing and approving non-clinical safety and pharmacology packages to support non-regulatory project decisions and regulatory submissions. Dr Davies holds a PhD in Rheumatology and Immunology from University of Birmingham, UK

PELIN CANDARLIOGLU PhD, is a tissue engineer by training having received her PhD in the field from Imperial College London but moved into oncology during her PostDoc position about circulating tumour cells at the UCL. Her introduction to organ on chip (OoC) was during the time when she was leading a Cell Biology/Microfluidics lab in Cambridge at Enplas Corporation where she was developing a microfluidic chip system specifically designed for immuno-oncology applications. Currently, as part of Complex *In vitro* Models (CIVM) group, she is leading a small team utilizing her expertise in microfluidics, tissue engineering and especially OoC to lead multiple initiatives both externally and internally to expand the complex *in vitro* model portfolio of GSK for immuno-oncology. Pelin is very active at the 3Rs initiative in GSK and supporting from reduce and replacement aspect. She is also representing GSK globally in relevant organizations such as NA3RsC MPS Initiative, IQ-MPS, NC3R, OOACT in UK and as chair of industry advisory board at EUROoCS.

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DOI: 10.18609/cgti.2022.002

Q What are you working on right now?

MD: I work in the Oncology Cell Therapy unit at GSK. This research unit is responsible for identifying new targets and cell therapy programs that could potentially lead to the development of a new medicine to treat patients with cancer. It is also responsible for developing and implementing new technologies to improve current approaches used in the clinic. The unit also supports progression of clinical assets by addressing scientific questions posed by the regulatory agencies.

JE: I lead the complex *in vitro* model (CIVM) group, which supports GSK from early discovery (i.e., from target ID through to candidate selection) and even to the stage where some programs are in the clinic. We are developing complex *in vitro* models that can be as simple as spheroid models all the way up to organ-on-a-chip models.

Currently, we support the immunology research units where we are developing epithelial models with an immune component. In oncology, we are developing models that have either the non-vascular or vascular component. This helps us understand the different levels of complexity of the tumor microenvironment in solid tumor, with or without the flow component.

Ultimately, we will have to decide where we want to move in terms of models that have an autologous system – for example, we could have tumor organoids with an immune component that are autologous. What we really want to achieve is using patient tumor cells in various settings.

PC: At a higher level, we are looking to all modalities, not only small molecules. I am very pleased that we are also looking at cell therapies as this is the up-and-coming model for many disease areas, not only oncology. We are looking to support the oncology cell therapy research unit at every step of the drug discovery workflow, starting from target identification to preclinical development.

Q What would you frame as the current state-of-the-art in organoid, tissue-on-a-chip, and other emerging *in vitro* tool innovation – what do you regard as the cutting edge, particularly in terms of potential to provide clinically relevant insights?

MD: We have been using patient-derived cancer cells and patient-derived xenograft cells. However, they have a number of shortcomings in terms of complex cell cultures such as their spatial organization due to cell dispersion steps. They are also expensive, and they have a low transplantation rate in animal models.

I think we are going through an exciting time with the development of organoids and tissue-based chip platforms that are combining different cell types within a given model. They closely recapitulate the tissue architecture, which then better resembles the complex tumor microenvironment.

We are also starting to combine innovative technologies with organoids, such as 3D bioprinting, or biomaterials and imaging. This enables us to improve, control, and compartmentalize different cell types, leading to better development of more suitable models that are going to reflect the tumor microenvironment.

PC: At the cutting edge of modelling tumor therapy is the creation of the physical and biochemical barriers that we see on tumor microenvironment for cell therapy applications.

Also, preserving patient-to-patient diversity is the key. It is not always possible to combine all of these aspects in the same model, but on the rare occasions that it is possible, and it all comes together, then we can describe it as cutting-edge technology.

JE: One of the exciting things we are looking at is creating models that represent the tissue in a more contextual aspect – for example, where we have the correct orientation in terms of the apical to basal polarity of certain proteins that are being expressed in the tumor. Also, the expression level of the antigen that we are targeting would be representative of what we would observe in a tumor versus a healthy cell. This will then allow us to look at the efficacy of the CAR-T cell, for instance.

Q How and where are these tools being applied at the moment to the greatest benefit?

"Having microphysiological systems (MPS) models gives us the confidence to tease out these targets in a human *in vitro* system before going to the clinic, helping to change our perspective and encouraging us to look at targets that we might otherwise consider to be too risky."

MD: In the research unit, they are critical systems that allow us to model target expression in its native state. Particularly for programs where we have targets expressed in tight junctions, these systems are much better than the 2D models, because we can better assess both target expression and accessibility.

We are also using the tools to investigate combination therapies where we can use a small molecule or a biopharma drug alongside a T cell therapy, with the aim of overcoming the immunosuppressive features of the tumor microenvironment.

JE: One additional aspect is understanding the trafficking of immune cells. By using flow-based models rather than static systems, you can start to understand the movement of CAR-T cells across the vasculature wall towards the tumor, as well as the interaction of the stroma with its inhibiting action and the T cell breaching the tumor. This is exciting as you can observe in real-time the movement of T cells and the destruction of tumor cells.

PC: These complex systems can bring the greatest benefit at preclinical development of cell therapies, particularly in the area of safety.

There are two main types of safety concerns: on-target and off-target. For off-target, our toolbox is fairly good, but when it comes to on-target, off-tumor safety concerns, things are slightly more complicated. This is because we are increasingly looking at specific human targets, it is becoming more challenging to pick these out because of the range of expression of the target overall. This also affects the way targets are selected from the beginning. Even though we are developing cell therapies that are showing success in the clinic, we currently lack full confidence in our preclinical models. As such, we are forced to make decisions that is considered only the safer targets, in terms of tissue expression levels and so on, whereas there is actually a whole range of targets that have the potential to be excellent, especially with solid tumors.

Having microphysiological systems (MPS) models gives us the confidence to tease out these targets in a human *in vitro* system before going to the clinic, helping to change our perspective and encouraging us to look at targets that we might otherwise consider to be too risky. In addition, our cycling times would be faster if we had better validated models that we can trust. This means that we can choose riskier targets and we could consider the move

DOI: 10.18609/cgti.2022.002

to the clinic much sooner because we can predict the clinical situation better. This would be the main benefit these MPS systems could offer to the whole field.

Q Where is progress being made in PK/PD modeling as applied in the cell/gene therapy field?

MD: PK/PD modelling is a particularly challenging area for cell therapies because the product is live; the cell phenotype and cell characteristics are constantly changing as the cells interact within the microenvironment. Some cells might die whilst other cells proliferate and expand.

We can measure CAR/TCR-T cells and their kinetics using methods such as quantitative PCR (qPCR). There are a few publications on the use of clinical and PCR data to model PK parameters and potentially guide dose selection for later-stage clinical trials. These models are still relatively nascent and will require clinical validation before they can be commonly used.

In the preclinical space for T cell therapies, we are using *in vivo* models. However, these often use immunocompromised mice, which lack key components of the human immune system. In fact, any mouse model, including humanized mouse models, are currently quite poor because we still don't understand the innate cellular kinetics in order to be able to extract their full translational potential.

For TCR-T cell therapies, we don't tend to run animal models routinely, because you need the peptide being presented on the major histocompatibility complex (MHC). For these therapies, RNA and protein expression levels of targets on cells can be used to understand exposure-response relationships. However, what we can do in terms of modeling PK is still quite limited in the classical sense – again, mainly because of the inherent complexities associated with cellular kinetics.

PC: PK/PD in general is definitely an area where there is a huge gap, but even more so for cell therapies. We are also looking at whether organ-on-a-chip can help. However, the current feeling in the field is that even if you were to use body-on-a-chip models, understanding complete PK/PD of a cell therapy is probably still too optimistic. It is possible to study exposure distribution levels using body-on-a-chip because it is all human cells and therefore, potentially more representative than what you would see in an animal model. However, there are overall many challenges to get to that point and there isn`t anything, to the best of my knowledge, that is published on PK/PD models using body-on-a-chip with cell therapies so far.

JE: One additional aspect is having vascularized tumor models where you can look at the number of T cells or CAR-Ts that are reaching the tumor. This could be understood by extrapolating the effector: target ratio. Obviously, in the initial exposure, it has to be a cellular product and not a biopharma or small molecule drug – you can potentially then get continued exposure due to the T cells being long-lived.

Q Can you speak further to the challenges or obstacles facing the cell and gene therapy field's adoption and utilization of novel *in vitro* and in silico tools?

MD: One of the challenging aspects is the variability and reproducibility observed across *in vitro* models.

It is important to find ways to evolve *in vitro* models to become more reproducible; this is particularly challenging, especially when we know that there is a lot of heterogeneity within the patients' own tumors. Additionally, the most popular models don't recapitulate the cells that are in the tumor and the immune system, so we can't necessarily reconstitute the microenvironment at the moment. That makes it difficult to predict clinical outcomes or define novel biomarkers.

PC: I feel the main issue is confidence to the models. The reason for this is the nature of the modality itself.

When we look at MPS models, we look at the clinic and try to reproduce the human response *in vitro* to validate and create confidence in the model. But when we are looking at cell therapies (and especially, in solid tumors) it is very challenging to find clinical success stories that we can replicate in order to better translate and validate our MPS model. We have to try to create, qualify, and validate our models in the limited ways that are available to us due to lack of clinical example data. It is not going to be as strong as it would be with certain modalities like small molecules and certain diseases where there is a whole lot of clinical history that we can try to replicate. And when that confidence does not yet fully exist, it is of course more challenging to apply in your workflow.

JE: Furthermore, most of the Complex *in vitro* models for cell therapies have longer cell culture periods than a normal 2D model, where cells are culture for hours, but CIVMs are still only cultured for days – so they are still fairly acute. We don't have standard culture models that go on for weeks or months, for instance.

Q Can you expand on the key issues relating to the current degree of standardization in the manufacture and usage of *in vitro* models?

MD: For me, the main issue is validation of the tissues that are selected to start your culture systems and the genetic changes that might occur after biopsies.

"It is important to find ways to evolve *in vitro* models to become more reproducible; this is particularly challenging, especially when we know that there is a lot of heterogeneity within the patients' own tumors."

DOI: 10.18609/cgti.2022.002

It is choosing the optimal biomaterial and the right media, which is true for any of the culture systems, but it's also in the microfluidics and understanding the sheer stress and oxygen levels.

Every *in vitro* model is going to need its own validation and part of that is thinking about how to measure the tissue function – what exactly is the readout that tells us if a system is truly representative of the real situation?

JE: We have been comparing some models, both in-house and with external collaborators, in an effort to find a good reference material in the cell therapy context. This would give us good positive and negative controls for specific targets that we would use as a test case. Having good reference standards is a critical aspect as well.

PC: In terms of manufacturing, technology developers are responsible for the standardization of the end product. The main players form the first generation of technology developers have already reached a certain maturity that they can offer standardization in terms of their manufacture.

From our perspective, we are looking for a functional standardization – a fundamental characterization of the system so that we can compare different platforms and models. That requires a reference point, a baseline created via positive and negative controls which can be different between modalities and the question asked. When you are looking for cell therapies, finding these positives and negatives tool compounds are not always very clear.

JE: It's important to note that in the CD19 space, there is already a lot of positive clinical data in relation to liquid tumors. However, the solid tumor space is still evolving.

Q What promising efforts are underway to address any shortfalls regarding standardization – and what more needs to be done here?

PC: Europe has some ventures that are addressing this currently; led by the European Joint Research Center of the European Commission, in collaboration with European Standardisation Organisations CEN and CENELEC. There was an excellent conference in 2021 called "Putting Science into Standards", with the specific focus being on organ-on-a-chip technology. The aim was to find out what the needs are for standardization of the organ on chip field in general. I was lucky enough to be part of this initiative.

Based on that conference, and particularly from an EU perspective, there is a clear recognition that the field has matured to a certain degree. We are now trying to understand how standardization will progress that to the next level. This involves standardization at every level (e.g., materials, reference points, context of use, validation, reporting, data management, and so on).

It is a big initiative and the chief purpose of this initial conference was simply to understand the needs of the various parties involved (e.g., the regulators, the end users, and

technology developers) and the main bottlenecks. This will hopefully lead to something being created by consensus that everyone will agree to and follow.

JE: In terms of other initiatives, there is the National Institute of Standards and Technology (NIST) in the USA, which is interested in looking at standards in the MPS area. They have already created some standards in the cell viability space for cell and gene therapies. I think they are now starting to look at what potential options exist to create standards for MPS through collaboration and consortia.

Q Turning to the Innovation and Quality Microphysiological Systems (IQ-MPS) affiliate consortia, specifically, can you firstly give us some background on how you came to be involved?

JE: When I joined GSK more than five years ago, Brian Berridge was the point of contact in the IQ MPS. It was through his leadership that we spun out and became the IQ MPS affiliate. I've been involved for the last four years, becoming Chair in 2021.

There are twenty-one pharma companies involved in the consortium. I see it as a good opportunity for pharma to come together as an industry and align ourselves on issues, even just to find agreement on the definition of MPS, which might seem trivial but isn't, in fact.

It also gives us the opportunity to talk about potential opportunities for data sharing, which is a major challenge. We are also able to have a more singular voice in the regulatory space when engaging with the FDA and other agencies around the globe. We can also look to have strategic partnerships with organizations such as EUROoCs, NC3Rs, NA3RsC, and NCATS at the NIH. These different organizations all want to be involved in driving the MPS space.

Q What are the IQ-MPS affiliate's chief activities, what progress has been made to date, and what are its key goals moving forward?

JE: We currently have five different workstreams that are linked with the goals for the IQ MPS – primarily, we want to be a thought-leader in the space, provide a venue for appropriate cross-pharma collaborations, focused regulatory agency interactions, and external partnerships.

One of these workstreams is in the organotypic manuscript area (www.iqmps.org). Currently, we've had nine manuscripts and we will be putting together another eight or nine in the future. This initial batch of manuscripts addressed different organ specific

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DOI: 10.18609/cgti.2022.002

industry requirements for MPS platforms. For example, the liver paper focused on how you would characterize and validate the liver, specifically in the safety/toxicity space. We have also done this for multiple other organ systems. This has been helpful for tissue developers and academics in this area, so they understand what we are interested in.

The second workstream is in the regulatory space. We have had ongoing dialogue through a number of webinars and workshops with the FDA to build alignment in our thinking around MPS and how it could impact drug discovery. A recent article in ALTEX illustrates these efforts [1].

We also have a number of early proof of concept projects ongoing – one is in the intestinal space and a second is in the kidney area. We see that as a crucial aspect where we can align on different endpoints, on different ways to characterize the models, and on specific context of use.

Another area of focus is strategic partnerships; we are looking to make sure we are aligning with different parties such as NCATS.

The final workstream focuses on the overall MPS landscape. We recently conducted a survey and will create a manuscript from the results on the use of MPS in pharma, focusing on the different contexts of use. That will be a really interesting paper to show where and how different companies are using MPS in practice.

PC: As part of the organotypic manuscript series we have a cell therapy paper in preparation, looking into where MPS can be used to inform better decision making. I am very lucky to be leading that initiative together with my colleague from AstraZeneca, Louise Delsing. We are looking to create a snapshot of the cell therapy field: what is available? Where are the gaps? Where do we think MPS will provide the biggest value? And how do we think MPS models need to be qualified and characterized when they are applied to cell therapies? We have very experienced colleagues from the IQ MPS, and so we are hopeful this paper will be a consensus paper within the cell therapy field, which will generate some guidance for all interested parties, including regulators.

MD: From the research unit perspective, we are supporting the work that Pelin and Jason are doing through these initiatives in order to be able to do things like supply reagents, or even have discussions about how the model would represent a proper physiological status. We bring that intellectual element about the disease and the reagents that are available to allow us to start to develop relevant suitable models.

Q Where specifically would you like to see further Working Groups like this one focused in future?

JE: Outside of IQ MPS, the one we discuss most is in the standards space. Pelin talked about the standards that are being discussed in Europe, led by EUROoCs and others. In a global setting, the FDA would be involved as well as other global regulators, of course, plus IQ MPS. Hopefully, we can start to come together to understand and develop a framework

from technical, biological, and also data aspects. This is crucial when extrapolating things to the clinical setting.

Q Finally, can you share your respective visions for the future application of these tools and the impact they can make on the cell and gene therapy space, and on biopharma in general?

MD: For me, the future will be about increasing access to these tools and that includes taking cost into consideration. I would like to see these systems used to model different disease pathophysiologies (e.g., metastasis), various tumor microenvironments, and perhaps even modeling the dormant or inflamed nature of cancers. I also see them being used as a more high-throughput screening mode to select targets.

Overall, things are going in a great direction. We have made fantastic progress in the field these last few years and I can see these models being used more and more as we start to better understand translation to the clinic.

JE: In terms of patient stratification, you could use models in the future to better define the direction you want to take in your clinical trials. We don't have the bandwidth at the moment to have large enough biobanks to look at different patient populations in addition to studying efficacy and safety.

PC: They will improve the identification of different targets. Well-validated targets will increase our confidence and ultimately, improve cycle times.

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DOI: 10.18609/cgti.2022.002

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *All authors are employees and shareholders of GlaxoSmithKline (GSK). Pelin Candarlioglu is a Chair of Industry Advisory board of EUROoCS and a member of NA3RsC MPS Initiative Leadership Team. The authors declare that they have no other conflicts of interest.*

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

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Interview conducted: *Nov 8 2022;* ***Publication date:*** *Feb 3 2022.*

INTERVIEW

Keys to the successful preclinical-clinical translation of next-gen CAR-T cell therapy

DEVON J SHEDLOCK, PhD, is Chief Scientific Officer, Cell Therapies at Poseida Therapeutics, Inc., a clinical-stage biopharmaceutical company utilizing proprietary genetic engineering platform technologies to create cell and gene therapeutics with the capacity to cure. He joined Poseida as its first employee in 2015 and most recently served as Senior Vice President of Research and Development. Dr Shedlock is a key scientific contributor in the application of the Company's proprietary gene engineering platform technologies to develop novel cell therapy programs. Before joining Poseida, he held positions as an adjunct assistant professor of pathology and laboratory medicine at the Perelman School of Medicine and associate director of the T-Cell Engineering Laboratory that is part of Carl June's group, both at the University of Pennsylvania. Dr Shedlock received his PhD in cell and molecular biology from the University of Pennsylvania, and a BS with Honors in biology from Ursinus College in Collegeville, Pennsylvania.

Cell & Gene Therapy Insights 2022; 8(1), 29–38

DOI: 10.18609/cgti.2022.004

Q Firstly, can you introduce us to Poseida Therapeutics' platforms and pipeline?

DS: Our mission at Poseida is to utilize our proprietary genetic engineering platform technologies to create differentiated cell and gene therapies. We are focused on developing new therapeutics with the potential to deliver single treatment cures for cancers and rare genetic diseases. Our technology enables a non-viral approach that is truly novel and gives us the potential to achieve better patient outcomes with lower toxicity and a promising safety profile, while maintaining effectiveness. Our leading candidates in cell therapies include off-the-shelf CAR-T products in both hematologic and solid tumors, and we are developing treatments for patients with multiple myeloma, prostate cancer, and soon others like breast, ovarian or lung as well. In gene therapy, we are working to address liver-related congenital genetic diseases with unique gene insertion, editing, and delivery tools that further set us apart.

Q Can you tell us about the opportunity for CAR-T in solid tumors, as you see it? And what are some of the key translational R&D challenges in pursuing it?

DS: I see a great opportunity in solid tumors for CAR-T. Historically, CAR-T therapies have performed extremely well against heme malignancies, but solid tumors have been challenging for a variety of reasons, including complex tumor architecture, immunosuppressive microenvironment (low O_2, acidic, nutrient dessert, etc.), high target heterogeneity within tumor, few 'clean' surface-expressed CAR targets, etc. For the most part, responses in the clinic have been poor, although there have been sporadic complete responses (CRs) requiring multiple doses of the product. To me, this is great news because it serves as proof-of-concept (POC) that CAR-T *can* be effective against solid tumors. It also shows that T cells, properly engineered and under ideal conditions, can achieve safe and meaningful responses in this challenging setting. In addition, response rates are only likely to increase when CAR-T therapy moves earlier in the standard of care, when patients are less 'beat up' by numerous treatment regimens, rendering them potentially more receptive to therapy in general, both autologous (auto) and allogeneic (allo), as well as yielding higher quality CAR-T products in the auto setting.

> "Our technology enables a non-viral approach that is truly novel and gives us the potential to achieve better patient outcomes with lower toxicity and a promising safety profile, while maintaining effectiveness."

I believe some of the key translational R&D challenges in pursing CAR-Ts in solid tumors are further engineering and creating ideal conditions for these cells to perform safely and optimally in patients. Dogma in

DOI: 10.18609/cgti.2022.004

the field states that CAR-T will need some type of 'armor,' such as 1) the expression of null or switch receptors, homing molecules, co-stimulatory receptors, secreted checkpoint inhibitors, activation or triggered gene expression systems 2) gene editing of the T cell targeting disruption of checkpoint molecules or genes associated with differentiation and/or 3) conditioning the CAR-Ts during manufacture via the addition of different cytokines, chemicals, and/or pharmacologic reagents. Each of these strategies aims to enhance performance of CAR-Ts in the highly immunosuppressive solid tumor microenvironment. However, each one faces key translational challenges that may add significant complexity, time, and/or cost to manufacturing. For example, cargo capacity of widely used viral vectors (e.g., lentiviruses and gamma retroviruses) is extremely limited and does not accommodate the delivery of larger transgenes. The addition of a gene editing technology can be a serious safety consideration as it increases the risk of genotoxicity that may lead to transformation and/or dysregulated activity. GMP quality supplemental reagents may be expensive and/or proprietary.

At Poseida, we recognized these key translational challenges at an early stage and are addressing them using our suite of non-viral technologies and proprietary processes. Our piggyBac® (PB) DNA Delivery System enables the stable integration of transgenes far greater in size than do viral vectors. Our Cas-CLOVER™ (CC) Site-Specific Gene Editing System provides a far safer and efficient way to knockout (KO) genes, such as during allo CAR-T manufacture. Importantly, both these technologies work together in resting T cells, which significantly reduces the possibility of genotoxicity and better preserves a desirable early memory phenotype of T cells. As such, we believe these key advantages may obviate the need of an 'armor' to achieve meaningful responses in solid tumors – specifically, if a product is rich in early memory CAR-T cells, primarily stem cell memory T cells (T_{scm}) – more on that below. Now, to be clear, that's not to say that further engineering with an 'armor' or pre-conditioning during manufacture won't improve the performance of a CAR-T_{scm} product. I'm just saying it may not be critical for better responses in solid tumors when treating with this early memory product.

Q Tell us about the importance of T_{scm} – why is having a high percentage of T_{scm} cells correlated with antitumor efficacy?

DS: T_{scm} are an ideal cell type for adoptive cellular therapy since they are long-lived, self-renewing, and multipotent. In general, the establishment of T cell memory is essential for long-term health and protection against infection as well as cancer cells. Most scientists believe the T cell differentiation pathway is essentially unidirectional and irreversible (Naïve > T_{scm} > Central Memory > Effector Memory > Terminally Differentiated Effectors). During this process, T cells gradually acquire increasing effector function as their DNA is epigenetically modified and chromatin is remodeled, but this is also associated with a progressive reduction in their capacity for proliferation and self-renewal, or 'stemness'. T_{scm} cells circulate through the blood at very low frequencies and spend most of their time in the lymphatics and associated organs. Importantly, they are thought to persist for decades (or more) maintaining the capacity for long-term cell-mediated immunity, e.g., as reported to occur after infection with yellow fever virus or other pathogens.

A CAR-T product rich in T_{scm} can be thought of as more of a prodrug, whereas traditional products comprised of more differentiated cells are a drug. As such, CAR-T_{scm} cells are considered safer since they possess limited effector function and must first expand to give rise to effectors, thereby requiring more time to become functionally active (i.e., the prodrug yielding the drug) and possibly reducing the risk of early cytotoxic events. Indeed, in both of our clinical trials we have observed delayed CAR-T expansion peaks (or C_{max}) in the blood of patients as well as significantly lower incidence and severity of CAR-T mediated toxicities such as cytokine release syndrome (CRS) and neurotoxicity. This safety profile has allowed us to treat many patients on an outpatient basis. Also, CAR-T_{scm} cells may be more effective since they can give rise to wave after wave of effectors *in vivo*, as needed, a capacity of which is likely far greater than that of products comprised of more differentiated T cells. Thus, this prodrug is dose-sparing, and a single dose may be equivalent to infusing multiple doses of a traditional CAR-T product. Indeed, we and others have reported correlations between best overall clinical responses (BOR) and the percentage of T_{scm} in the drug product. And now, most recently, we've observed several PSA50 (prostate specific antigen declines of at least 50%) responses and one patient demonstrated evidence of complete tumor elimination early in our P-PSMA-101 Phase 1 trial for prostate cancer, the latter of which to our knowledge is unprecedented for a single dose of a CAR-T. Lastly, a CAR-T_{scm} product may be more durable since these early memory cells are long-lived. In fact, once tumor burden is reduced or eliminated and effector CAR-Ts have contracted, a population of CAR-T_{scm} likely persist to provide protection against possible tumor relapse. We have now observed this phenomenon both in preclinical tumor-bearing mouse models and in the clinic. Thus, as a prodrug, a CAR-T_{scm}-rich product may be considered a lot like a vaccine that provides protection for a long period of time. It is for these reasons I believe T_{scm} may be the key to greater safety, efficacy, and durability in the clinic.

How is a CAR-T_{scm} product made at Poseida? Ultimately, I believe the phenotypic composition of a CAR-T product is determined, in large part, by the type of gene delivery vector used during manufacturing. Our nonviral PB and proprietary manufacturing process generates allo CAR-T products with exceptionally high T_{scm} percentages, sometimes as high as 80%. For reference, most competitors using lentiviruses have reported CAR-T_{scm} levels from 0% to 12%. What may possibly explain these stark differences? To answer this question, we performed a simple experiment to determine how well each delivery vector modifies the different T cell subsets. While PB demonstrated the highest levels of transposition into naïve and T_{scm} cells, lentivirus preferred to transduce central and effector memory T cells, but not naïve and T_{scm} cells. So, if your product's functional capacity is dictated by its phenotypic composition, and you can't likely do better than the T cell subsets initially modified since the T cell differentiation pathway is one-way, then your gene delivery vector may ultimately define your product. Essentially, your process is your product. Thus, if you use virus, you will likely need to pursue other avenues to further

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DOI: 10.18609/cgti.2022.004

improve the safety, efficacy, and durability of a differentiated product – see key translational R&D challenges above. With that said, I do believe any CAR-T product could be improved, at least phenotypically, by preventing or slowing further differentiation during the manufacturing process. To this end, we use a proprietary expansion medium and do not add any cytokines. In addition, our PB and CC technologies work in fully resting T cells, which we think reduces possible differentiation, whereas most viruses and some gene editing technologies require the cells to be first activated and dividing to be permissive to transduction and editing, respectively. Both of our platform genetic engineering technologies, we believe, help to preserve early memory cells during manufacture and enhance a favorable phenotypic composition in the final product.

Q Poseida is advancing a largely allogeneic cell therapy pipeline towards and into the clinic – how is Poseida's technology/approach suited to driving the development of allo product candidates in particular?

DS: At Poseida, we believe allogeneic CAR-T is the future. Working towards this goal, we have leveraged our learnings and clinical experience from our first two auto CAR-T programs (P-BCMA-101 in multiple myeloma (MM) and P-PSMA-101 in prostate cancer) and developed a safe and efficient platform and process using our core technologies as described above. Specifically, co-delivery of PB and CC during manufacturing allows for simultaneous CAR transgene integration and targeted disruption of the T cell receptor (TCR) and human leukocyte antigen (HLA). The first gene edit is critical and required to eliminate the possibility of TCR-mediated graft-versus-host disease (GvHD). We then eliminate the great majority of remaining non-edited TCR-positive by purification. The second edit facilitates engraftment in HLA-mismatched patients by eliminating the potential for T cell-mediated host-versus-graft rejection. However, in this case, we aim for approximately a 50% HLA KO rate and do not purify cells based on major histocompatibility (MHC) expression since little clinical data for allo CAR-Ts are available demonstrating which population is more effective in patients. Thus, with this mixed approach we can monitor both populations *in vivo* in real time to learn more about their engraftment kinetics as our trials progress. Lastly, we developed a proprietary booster molecule that facilitates generation of potentially hundreds of doses per manufacturing run, effectively avoiding the dreaded 'Allo Tax,' a term coined by others in the field that refers to poor expansion and function of gene-edited CAR-T products in general. (See more about our booster molecule and how it works below).

From a safety perspective, both PB and CC provide some strategic advantages in the production of allo product candidates. As mentioned above, both technologies work in resting T cells, which we believe significantly reduces the chances of genotoxicity. As such, the potential for insertional mutagenesis of cell cycle genes that drive proliferation may be reduced or eliminated in resting cells. On the contrary, lentiviruses require the cells to be activated and expanding to be permissive to viral transduction. In addition, PB has been reported to be far less (~40% less) intragenic than lentivirus. Regarding CC, we think the capability of gene editing in resting

cells is considerably safer since the possibility of genomic instability and chromosomal abnormality is increased when double-strand breaks (DSBs) are not repaired prior to chromosomal segregation during cell division. However, this might not be possible for manufacturing processes that couple gene editing with lentiviral transduction in activated cells. This also may be the case with other gene editing technologies that don't seem to work efficiently in resting T cells, such as TALENs. Indeed, overall translocation rates in our allo CAR-T products were up to 10-fold less than those published for CRISPR and TALENs (<0.4% for CC as compared to 2–4% for CRISPR and TALENs). And our average rate of translocation with off-target sites was less than 0.01%. Thus, we believe we have some key strategic technological advantages particularly suited to safety, efficacy and driving the development of allo product candidates.

Q What are your thoughts on how the field can seek to address a current lack of good non-clinical models for allogeneic cell therapy development?

DS: I still believe that *in vivo* models are the best for cell therapy development. I'm talking about your average, run-of-the-mill tumor-bearing NSG mouse models. After a tremendous effort, we found no *in vitro* assay that comprehensively assessed the quality or fitness of a CAR-T product. Sure, most assays can tell you something informative about your cells, like if the CAR is expressed on the cell surface, are the cells specific for their target, can the CAR-Ts kill, do the CAR-Ts express multiple effector functions, etc. And this can certainly be useful for screening purposes wherein experimental CARs or conditions that are particularly disruptive, or undesirable can be eliminated. But when it comes to identifying the best of several lead candidates that may show minor phenotypic or functional differences, nothing has served us better than assessing them head-to-head at low 'stress test' doses in tumor-bearing NSG mouse models. In fact, we've even had cases where products were essentially indistinguishable via all standard *in vitro* assay analyses but performed dramatically differently *in vivo*. Thus, a model of greater complexity such as an *in vivo* system that can bear an established tumor along with its associated microenvironment, while not a perfect representation of a human tumor, is likely a far more relevant way to assess a product's quality than standard *in vitro* analysis.

Why is assessing the quality of a CAR-T product so important to us? Because we believe those products with the highest quality will be the most effective in the clinic. What do I mean

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by a product's quality? I see it as a biological measurement of, in part, functional capacity and metabolic fitness, which may be a measure of how well the cells will perform in a complex environment, likely determined by where those cells derive their energy (either from internal or external sources). In theory, these cells are healthy and hearty, harboring the greatest capacity for engraftment, trafficking, proliferative responses, survival, and persistence. Thus, tumor-bearing NSG models may, in part, recapitulate some of the complex immunological environments for these responses to occur. And this may be especially important for a T_{scm} product like ours, which is more of a prodrug that needs to first expand to give rise to the drug, or effectors, that ultimately kill tumor cells. Most *in vitro* assays just can't replicate those complex conditions where CAR-T cells are forced to navigate both host and tumor microenvironments. However, a possible exception for assessing the proliferative and killing capacity of a CAR-T candidate is the repeated stimulation/killing *in vitro* assay where the product proliferates in response to multiple tumor cell challenges at regular intervals. But functional assessment here is limited to fewer effector functions and while informative, may not be as complete or rigorous as the *in vivo* system.

Lastly, I do believe *in vivo* tox and humanized models are important to assess possible on-target off-tumor toxicity, GvHD, CRS, etc. There has been some advancement in the field for these, but most are still in development. Thus, it'll be interesting to see in which direction the field goes on these depending on their utility and predictive capacity.

Q You were at the Perelman School of Medicine and working in Carl June's team during a key period in the development of the nascent CAR-T cell therapy field – can you distill a few translational R&D learnings and best practices that you bring forward to Poseida from that experience?

DS: I gained an invaluable perspective and appreciation of the most significant challenges to developing safe and effective CAR-T therapies while working on Carl June's team. How can we reduce CAR-T side effects while making them more potent and durable, especially against solid tumors? I believed the problem was one of T cell engineering and likely stemmed from technological limitations. I wanted to enhance the capabilities and functionality of CAR-T cells by editing genes and delivering larger cargo. Lentiviral vectors just didn't have the capacity for efficiently delivering more than a promoter and a CAR. If only we could also deliver a safety switch, or a combination of antigen binders for multi-targeting, such as CARs and/or a CAR and T cell receptor (TCR) to potentially limit antigen escape by tumors, especially in solid tumors where heterogeneity is a challenge. Or additional technologies helping to overcome signals from the immunosuppressive environment such as null or switch receptors, an activation-induced gene expression system that could secrete a checkpoint inhibitor or chemokine at the solid tumor site, etc. On top of that, I knew that disruption of certain genes like checkpoint molecules may help to make the CAR-Ts more potent as well as to generate allo CAR-T therapies. From this experience and perspective, I believed the next

step in my career would enable me to tackle some of these significant challenges stemming from early technological limitations.

I jumped at the opportunity to join an exciting spinout company sporting both non-viral gene insertion and gene editing technologies as its first official employee in early 2015. Having both technologies under the same roof made Poseida feel like a true platform company where the therapeutic applications could be possibly limitless. As head of immuno-oncology tasked with hiring the first scientists and getting the labs up and running, I aimed to innovate as a means to work towards becoming competitive in the rapidly growing cell therapy field. It was this drive for invention that undoubtedly stemmed from my prior experience in the novel biotech-like environment at UPenn, where innovation and drug development experience came together to create the first FDA-approved CAR-T therapy. I am convinced this ground-breaking achievement was facilitated and accelerated by the many great people working together in partnership between academia and industry. The best of both worlds, if you will. In a very similar way, many of the first scientists at Poseida were from academia, hired from cutting-edge labs and research groups from across the country and brought together into the biotech setting. I knew we needed to think outside the box and having fresh perspectives from different backgrounds seemed like a great recipe for problem solving and advancing disruptive science. Why wait for our competitors to show us the way when we could innovate on our own? I believe this approach ultimately helped us develop our unique CAR-T_{scm} platform, among others, and shape Poseida into the thriving company it is today.

Q What are the most significant ways in which the preclinical and translational R&D 'toolkit' has evolved over the intervening years – and what would you pick out as the key tools that can help drive clinical success in solid tumors?

DS: As I mentioned above, having both gene insertion and gene editing technologies under the same roof was a major reason I came to Poseida. In the intervening years, we made numerous advancements to each platform along with developing several new ones. One of these is our proprietary booster molecule, which solved a fundamental problem in allo CAR-T manufacturing and helped advance our first allo CAR-T programs into the clinic. TCR gene-edited T cells often experience poor expansion during manufacture and may not work as well as their non-edited counterpart, a phenomenon coined by others in the field as the 'Allo Tax.' We interpreted this to possibly be a result of at least two different consequences of genetic editing during CAR-T manufacture:

1. KO of TCR typically leads to lower cell yields at harvest since most expansion reagents work by engaging said TCR. In other words, lose the TCR, lose the robust T cell expansion. And, in the allo business, you want as many doses as possible from each manufacturing run to treat as many patients as possible;
2. performing multiplex gene editing, i.e., making multiple on-target cuts in the genome, especially in dividing T cells, may increase the chance of genotoxicity and may also negatively affect T cell biology and function.

DOI: 10.18609/cgti.2022.004

Thus, problem #1 was solved by inventing our proprietary booster molecule, while problem #2 was addressed by PB and CC having exceptional safety profiles, as well as working efficiently in resting cells.

The key tools helping us to drive clinical success in solid tumors are our core allo CAR-T platform technologies – PB, CC, booster molecule and our proprietary manufacturing process, and our learnings from our first two clinical trials evaluating our auto CAR-Ts in multiple myeloma and prostate cancer. To date, clinical findings for these programs have been incredibly promising and data have supported many of our initial preclinical hypotheses. For instance, we found that T_{scm} cells in the auto CAR-T product correlated with best responses in our MM clinical trial. They were also able to home to bone marrow, engraft, persist in patients for long periods of time (over 2 years in some cases), reduce the incidence of CAR-T associated toxicities, and even re-expand at tumor relapse after many months post-infusion (at 23 months in one patient). Interestingly, we observed similar finding in our non-clinical pharmacology studies in tumor-bearing mice where there were strong anti-tumor responses, the capacity for CAR-T persistence, and re-control of tumor in the event of relapse. And, as mentioned above, we've recently observed several PSA50 responses and evidence of potential complete tumor elimination early in our P-PSMA-101 Phase 1 trial. This latter patient had no evidence of tumor via bone marrow biopsy at a site of prior tumor involvement where CAR-T cells were detectable. We are tremendously encouraged and excited by these results and are already working on an allo version of the PSMA-targeted drug. Lastly, I must mention our newest solid tumor candidate, P-MUC1C-ALLO1, for the treatment of multiple solid tumors derived from epithelial cells such as breast, ovarian, among many others. Considering these powerful tools in our arsenal, we are extremely optimistic about our allo CAR-T platforms and how they may perform in the clinic in both heme and solid tumors.

Q Finally, can you sum up the major goals and priorities, both for yourself in your own role and for Poseida as a whole, over the coming 12–24 months?

DS: For me:

- Continue to learn, promote innovation, hire excellent team members, and aim to inspire
- Advance safe and effective pipeline programs to IND
- Help establish new technology collaborations or partnerships

For Poseida:

- Continue to advance new allo CAR-T and gene therapy programs into the clinic
- Further demonstrate efficacy and tolerability in solid tumors indications
- Establish new technology collaborations and partnerships

Our mission is to create the next wave of single treatment cell and gene therapies with the capacity to cure cancers and rare genetic diseases. I couldn't be more excited about our future.

AFFILIATION

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AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The author is an employee of Poseida Therapeutics Inc. with salary, benefits and equity. The author declares the following patents; Issued patent US10,329,543, Issued patent US10,415,016, Issued patent US10,513,686, Published patent WO2021183275, Published patent WO2021127505, Published patent WO2021046261, Published patent WO2020132396, Published patent WO2020077357, Published patent WO2020077360, Published patent WO2020051374. Various published and pending PCT and US patent applications (available upon request). All patent rights have been assigned to Poseida Therapeutics Inc and the author receives no royalties or other compensation for these patents outside of the author's compensation. The author declares that they have no other conflicts of interest.*

Funding declaration: *The author received a grant from California Institute of Regenerative Medicine (CIRM) - Grant investigator; https://www.cirm.ca.gov/our-progress/awards/late-stage-preclinical-study-car-t-memory-stem-cells-targeting-psma-p-psma-101. The author received Patent co-inventor (us WO2015112626) Royalties from Trustees of the University of Pennsylvania. The author received no other financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Interview conducted: *Dec 21 2021;* ***Publication date:*** *Feb 7 2022.*

DOI: 10.18609/cgti.2022.004

FEBRUARY 2022 Volume 8, Issue 1

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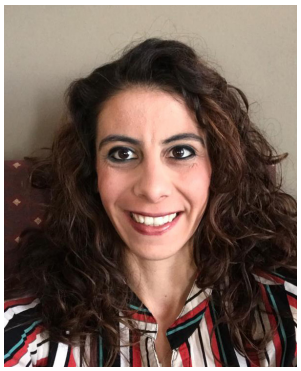
Residual DNA testing in viral vector manufacture: exploring the challenges and solutions



Cell & Gene Therapy Insights 2022; 8(1), 89–95

DOI: 10.18609/cgti.2022.026

Q IS: Mike, to begin, could you give a brief introduction and background on the residual DNA testing regulatory requirements in general, and why gene therapy products present some particular challenges?

MB: For recombinant viruses used in gene therapy treatments, there are additional challenges compared to monoclonal antibody (mAb) manufacturing and purification processes.

Specifically, the regulatory guidance on host cell DNA is that there should be less than 10 nanograms of host cell DNA per therapeutic dose. Additionally, at present you should be able to demonstrate that the DNA is less than 200 base pairs in length.

This guidance was essentially carried over from the older guidance on host cell DNA regarding manufacturing of cell-culture based vaccines. Some manufacturers that have been in that space may already be familiar with this guidance.

Additionally, for recombinant viruses such as AAV, there are multiple DNA residuals of concern depending on the processes. It can be host cell DNA, plasmid vector, or helper virus DNA that is part of the process. Multiple DNA assays may be required for full characterization at the levels and the capability of your purification process to reduce the levels of these DNAs.

You also need to have a good size assessment assay. DNA fragment size determination is expected, and that may need to be done at different points in the process, along with quantitation of the remaining host cell DNA at the end of production. In some cases the level of that DNA may be too low to enable accurate size assessment.

An additional challenge with AAV and host cell and vector DNA is recombinant AAV has been shown to encapsidate fragments of both vector and host cell DNA. The size of these encapsidated fragments can be significant; up to 5 kB. That can create challenges, including being able to reach that 10 nanogram per dose limit for high dosage formulations in these gene therapy applications.

Finally, if using cell lines that are known to contain potential oncogenes such as *E1A*, or SV40 T antigen in the case of 293-based processes, the presence of these genes and potentially the size of the genes that are present should be characterized.

These are all the additional considerations that manufacturers must take into account for these viral vector manufacturing processes.

Q IS: Before we analyze each of these particular concerns, let's discuss some of the challenges in the development and validation of residual DNA analytical assays in general, and in particular for gene therapy.

MB: Let's start with development. Development of these assays for sensitive and accurate quantitation of host cell DNA and other DNA residuals requires specialized expertise, it takes considerable time, and you have to develop multiple documents including

DOI: 10.18609/cgti.2022.026

method standard operating procedure (SOP) preparation qualification of critical reagents, equipment SOPs, and development and qualification reports for the new method. Plus validation protocols, execution of that validation, and generation of a report.

If an organization has the time and expertise, then certainly in-house development can be considered. However in this era they are fully integrated, and that includes sample preps, standard DNA, quantitation assay, even application-specific software and kit-based solutions for host cell and other DNA residuals. In the case of the Applied Biosystems™ ResDNASEQ™ kits, they are also supported by experienced application scientists and regulatory support teams. Therefore the process of implementation of a host cell DNA assay purchased from a vendor can be accelerated dramatically. A new user can be generating valid results in a few weeks, as opposed to the months or even years required for in-house development.

> "Development of these assays for sensitive and accurate quantitation of host cell DNA and other DNA residuals requires specialized expertise, it takes considerable time..."

My view on this, and I am sure it's shared by many others, is that if a high-quality kit is available for your application, then use it. Spend your time and resources on generating results, not on development of a method.

One other thing to keep in mind is that a rigorous, well-designed study to demonstrate a robust host cell DNA clearance and purification process can require analysis of a large number of samples. A method that can be automated, such as sample preparation, can be a huge value and efficiency driver.

The sample preparation method used in the ResDNASEQ kits is based on magnetic beads, so there are two options for automating that workflow. That is an important consideration as you look to the future.

Finally, moving to validation. For most applications, host cell DNA testing is considered a quantitative test for impurities and should be validated as such, per the ICH Q2 (R1) guidance on validation of analytical procedures.

Typically the test performed for a quantitative method should include accuracy, both repeatability and intermediate precision, limit of quantitation, specificity, range, and linearity. Robustness of a method should also be demonstrated, but it is acceptable to do that as part of method development – or that data can be supported by the vendor if you are using a solution such as ResDNASEQ. We have extensive data demonstrating robustness that was done as part of our method development process, and this certainly can be shared during a regulatory review.

Q IS: Looking at validation and qualification, how does the Thermo Fisher Scientific development validation study help ensure the quality of the kits, and how does this differ from the validation of the method for regulatory approval?

MB: As part of our development process we have a very well established and defined process. There are tests we put a new method through as we develop it and then as we get near completion, to demonstrate the robustness of it and assess how small deviations to the recommended method affect performance. We keep those all well documented, and as I mentioned before it can be available for referencing during a regulatory review.

When I use or hear the term qualification when discussing analytical methods, I consider that step as the initial demonstration, in a fairly rigorous manner, that the method will perform.

Can it accurately quantitate DNA recovered from key sample types, or from key sample matrices? Can it detect a contaminant in a key process sample? Qualification can also be referred to as pre-validation, generating a set of data that enables design of a validation study, and importantly, the health and setting appropriate acceptance criteria for that validation study.

Then importantly, the development stage of your manufacturing process and your clinical process should be considered; where and when use of a qualified method is acceptable for testing, and when a validated method is required.

It can be acceptable to use a qualified method for testing at the preclinical and early clinical stage of your product. Then, following success in advancing a product candidate through clinical trials, at some point validation will be required.

When you are selecting analytical methods, choose a method early that offers the performance required and looks able to be validatable, and critically, a method that typically regulatory have accepted in the past, following validation of submission and inclusion in your CMC package.

Choosing the right method early is critical so that you don't have to go back and redevelop or switch to a new method late in the process where there are lots of tasks that need to be accomplished in order to file an application for approval of a new product.

When working closely with an experienced vendor, key considerations should be that they can provide analyst training and workflow, equipment validation support, and provide examples of validation study design and drug master files when appropriate. The ability to support on regulatory use and having a record of success should also be key considerations when selecting an analytical solution. This is where Thermo Fisher in particular is very strong. In my opinion, the team we have supporting our products is unmatched in the industry.

In certain cases, such as here where we are talking about host cell DNA and other DNA residual testing with the ResDNASEQ product, the vendor can be a collaborator, and that will accelerate timelines and provide confidence in success.

"When working closely with an experienced vendor, key considerations should be that they can provide analyst training and workflow, equipment validation support, and provide examples of validation study design and drug master files when appropriate."

DOI: 10.18609/cgti.2022.026

Q IS: Focusing on gene therapy, and residual DNA testing in particular, let's address a couple of specific questions I am often asked by customers. How do we help simplify the process of measuring vector DNA, and how do we address the challenges of fragment sizing an oncogene?

MB: In the past people hadn't given as much consideration to testing for these DNA residuals, as well as sizing. To support that we have recently introduced three new products for recombinant virus manufacturing.

One of these is a combination SF9 and baculovirus residual DNA assay, and this is for insect cell culture-based manufacturing processes. We also now have an assay for residual vector DNA for vectors that are used in the recombinant AAV workflow, and this assay targets an element common in many plasmid vectors, the kanamycin resistance gene.

Finally we have an assay for detection of the *E1A* gene, that is present in 293 cell-based processes. Certainly that gene would be undesirable if it was present in the final product, but additionally the *E1A* assay enables size assessment of the *E1A* gene, and can also be used to assess the general size and quantity of host cell DNA fragments as you go through the purification process.

This is accomplished by use of a primer design for detection and quantitation of three distinct size classes of the DNA in the sample: the larger size of 476 base pair amplicon, an intermediate size of 200 base pair amplicon, and finally an assay specific for small fragments. This is important because this is an 86 base pair amplicon, which is below the regulatory guidance of less than 200 base pair fragments.

Q IS: Do you have any suggestions on how to address copy number versus mass concerns?

MB: For some analytes such as host cell DNA, regulatory guidance has always been the mass of the analyte in the sample. For other analytes such as the residual vector or *E1A* fragments, it may be more appropriate to report that result than copies of the analyte. The most important consideration here is to use a method that can accurately generate results and data that is aligned with the regulatory guidance for requirements. If you are using an assay like quantitative real-time PCR, the results can be generated and reported in either copy or mass of DNA, depending on how you design the experiment.

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AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The authors are Thermo Fisher Scientific employees.*

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

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Interview conducted: *Dec 16 2021;* ***Publication date:*** *Feb 21 2022.*

DOI: 10.18609/cgti.2022.026

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Optimizing downstream purification of high-quality plasmid DNA with POROS Chromatography Resins

Alejandro Becerra, Principal Applications Scientist and Global Purification Technical Lead, Thermo Fisher Scientific, and
Johannes F Buyel, Head of Bioprocess Engineering Department Fraunhofer IME Scientific Project Coordinator, Rubhu Biologics

The demand for plasmid DNA (pDNA) has increased in recent years, but due to their physical properties there are some inherent challenges to the purification of these molecules. A typical downstream process for plasmids normally has multiple steps after fermentation, and anion exchange followed by hydrophobic interaction chromatography are commonly utilized. Thermo Fisher Scientific has developed a variety of resins well-suited for these steps, designed to simplify workflows and increase purity and yield. A series of experiments were conducted in order to evaluate POROS™ AEX resins for pDNA capture, with the goals of optimizing process conditions to maximize purity and recovery, determining the dynamic binding capacity (DBC) of POROS AEX resins for pDNA, and confirming optimal operating parameters. Some highlights of these studies, performed in collaboration with the Fraunhofer Institute for Molecular Biology, Germany, are presented here. POROS™ D50, HQ50 and XQ were selected and evaluated for plasmid capture applications.

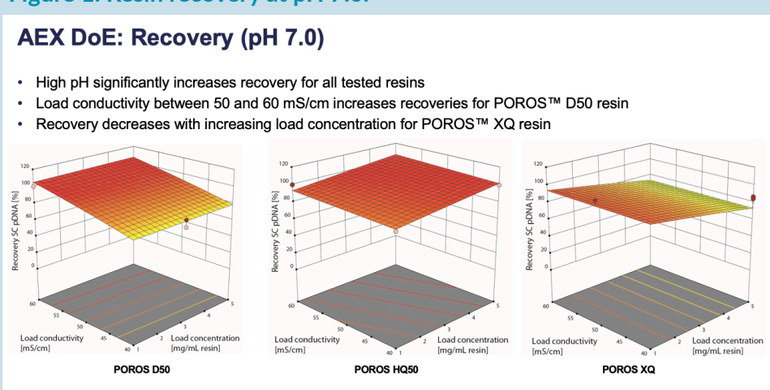
Cell & Gene Therapy Insights 2022; 8(1), 87; DOI: 10.18609/cgti.2022.029

PH AND PURITY

Overall recovery at pH 7.0 was fairly high **(Figure 1)**. Notably, for the POROS™ HQ50 resin, the different parameters had little effect; in this case the load conductivity and load concentration. In contrast, for POROS™ D50, we found that with an increasing load conductivity the relative recovery of products increased. For POROS™ XQ, the recovery decreased with an increasing load concentration, i.e., with a higher quantity of plasmid loaded per volume of resin. Using a pH of 6, this initial behavior was amplified. POROS HQ50 again showed relatively stable behavior throughout the design space.

Purity for all three resins was in a good range – between 60 and 75% of total nucleic acid was supercoiled pDNA, and conditions were identified that gave close to 100% recovery for all resins.

Figure 1. Resin recovery at pH 7.0.



DYNAMIC BINDING CAPACITY

The DBC of the different resins is an important question to address, as this will ultimately dictate the process economics. The D50 resin provided the highest dynamic binding capacity **(Figure 2)**, and was therefore the best suited resin to verify our results using a scaled-up version of the experiment.

POROS D50 SCALED UP VERIFICATION

Using a scaled-up experimental procedure we verified that the binding capacity was more than 10 mg/mL **(Figure 3)**. In the gel at the bottom of Figure 3, it can be observed that in addition to the plasmid in the different salt elution steps there is a fraction of product that is eluting only once the cleaning procedure is applied (seen on the right side of the gel, in the lane labeled with CIP). Therefore, it is likely that optimizing the current elution conditions can increase the recovery.

Figure 2. POROS™ D50 dynamic binding capacity.

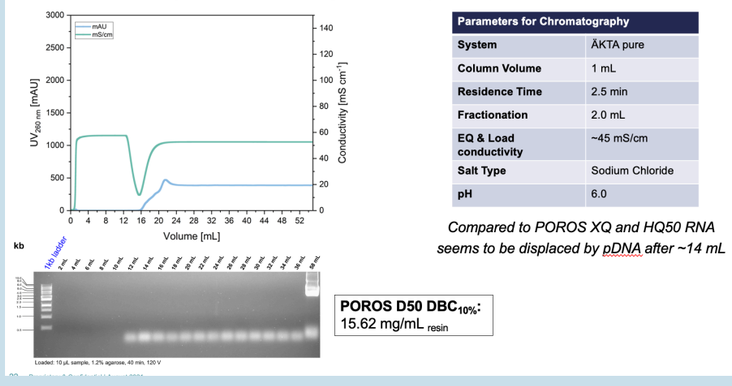
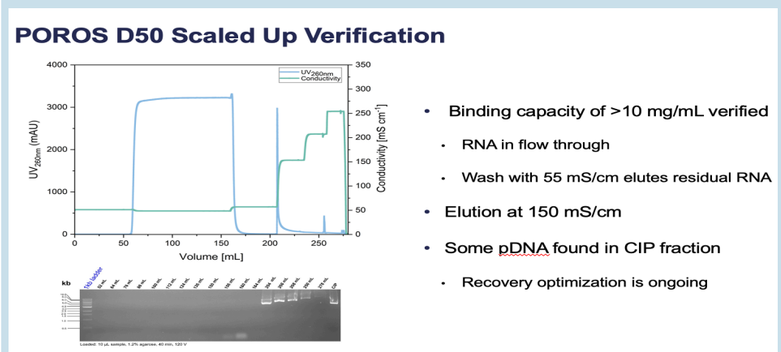


Figure 3. POROS™ D50 scaled up verification.



INSIGHTS & FUTURE DIRECTIONS

High binding capacity was obtained for all three resins, with POROS D50 demonstrating the best binding capacity. Residence time was 2.5 min, and increasing this may increase the binding capacity observed. Initial scale-up verification confirmed the high capacity, purity, and recovery for POROS D50, and work is ongoing to optimize the D50 capture step.

To explore the full study design & results, along with an author Q&A, watch the webinar or read the article

Read the full article here

In partnership with: ThermoFisher SCIENTIFIC

Accelerating downstream analytical testing for gene therapy

Harald Ehlen, Repligen Corporation

Currently, the most commonly used methods for adeno-associated viral (AAV) vector quantitative analysis are qPCR or ddPCR and ELISA assays, along with analytical ultracentrifugation (AUC) and transmission electron microscopy (TEM). However, the time to result when using these methods can range from a day to several weeks, and the acceptable tolerance range is high. Rapid, reliable in-process testing offers a significant benefit to AAV downstream process development, and can be achieved with Slope Spectroscopy utilizing variable pathlength technology.

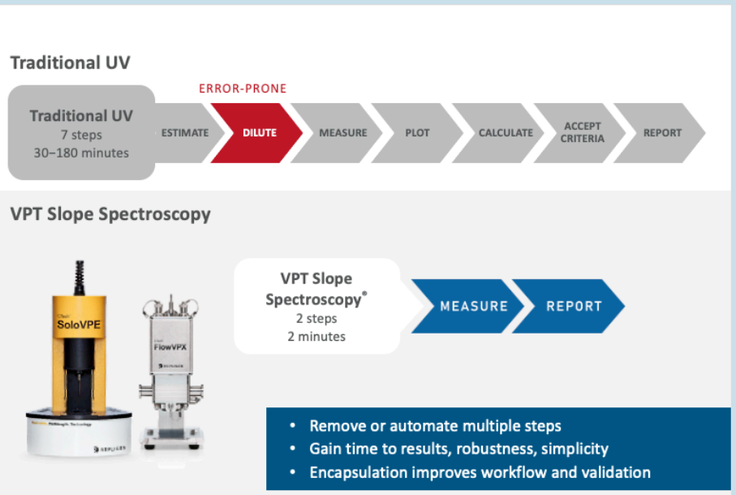
Cell & Gene Therapy Insights 2022; 8(1), 23; DOI: 10.18609/cgti.2022.025

INTRODUCTION TO SLOPE SPECTROSCOPY

Similar to traditional UV-Visible spectroscopy, Slope Spectroscopy is based on the Beer-Lambert law, which is expressed as: A = εlc. 'A' is the measured absorbance, 'ε' is the wavelength dependent molar absorption coefficient, "l" is the pathlength, and 'c' is the sample concentration. With Slope Spectroscopy, the pathlength varies, using variable pathlength technology (VPT), while the concentration remains constant – eliminating the need for sample dilution or manipulation. Data from up to 10 different pathlengths are used during the measurement to quantify the concentration with high accuracy, and data acquisition takes less than one minute.

With this VPT approach, process steps are greatly reduced, allowing for simplicity, speed, and more accurate measurements **(Figure 1)**. Adoption strategies can include analyses in the lab, at-line testing for improved process efficiencies, and in-line process analytical testing.

Figure 1. Slope Spectroscopy removes or automates multiple steps.



CTech™ SoloVPE® System for at-line testing; CTech™ FlowVPX® System for in-line testing.

GENE THERAPY CASE STUDIES: VIRAL VECTOR APPLICATIONS

The objective of this study, performed in collaboration with Vigene, was to demonstrate the CTech™ SoloVPE® System's ability to use the ratio of 280 nm and 260 nm slope value to make real-time decisions prior to subsequent analysis. Slope data obtained from measurements of AAV samples showed excellent linear regression data for both wavelengths with R^2 of at least 0.999 and %RSD of less than 2% proving excellent data quality.

Equivalency of both method results compared to current qPCR/ELISA method well within the +/- 40% range **(Table 1)**. Genome and capsid titers of Vigene Certified Reference Standards were measured within minutes versus days with current methods demonstrating the suitability of the CTech™ SoloVPE® System as a rapid at-line test for downstream process analytics in AAV process development and manufacturing.

In a separate case study, our in-line technology was used to monitor a UF/DF process. CTech™ SoloVPE® was used during a UF/DF step to measure AAV viral titer **(Figure 2)**. The results matched well to the ddPCR results gained by the customer after the UF/DF step. This allows accurate monitoring of this step in real time, allowing you to know the AAV titer without using complicated, and time-consuming, ddPCR method. This allows manufacturers to carefully concentrate up without compromising product integrity.

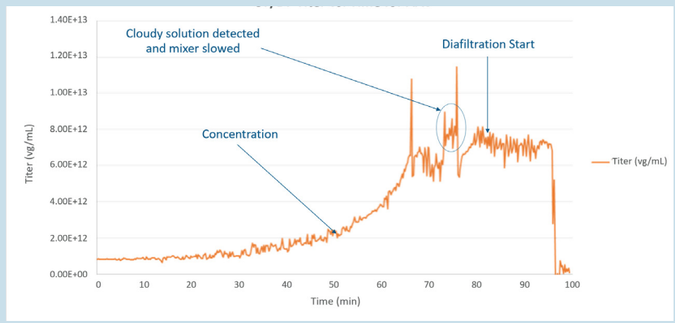
Click here *to learn more about variable pathlength technology and CTech™ Analytical Solutions.*

Table 1. AAV empty/full concentration determination.

Sample name	SoloVPE System			qPCR/ ELISA	% diff.
	DNA (vg/mL)	CAPSID (cp/mL)	% F/E	% F/E	
AAV8 empty	9.38×10^{11}	2.18×10^{12}	43.0%	15.4%	27.6%
AAV8 full	1.24×10^{12}	1.40×10^{12}	88.0%	74.8%	13.2%
AAV9 empty	3.00×10^{11}	4.59×10^{12}	6.5%	7.9%	1.4%
AAV9 full	7.51×10^{11}	9.81×10^{11}	76.5%	82.3%	5.8%

Acknowledgement of the Vigene team for AAV reference material production and testing:
Jian Zhang, PhD, Sean Kell, Mingjuan Lui, PhD, Audrey Chang, PhD, Cuiping Zhao, PhD, Jeffrey Hung, PhD.

Figure 2. UF/DF AAV titer versus time using the CTech™ FlowVPE® System.



	PCR (vg/mL)	Avg. FlowVPE System (vg/mL)	% diff.
Starting concentration	6.20×10^{11}	8.63×10^{11}	39.14%
Average diafiltration	7.70×10^{12}	7.20×10^{12}	6.45%
Final drug substance	5.20×10^{12}	5.51×10^{12}	5.94%

CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Magnetic selection for consistent cellular starting material in autologous cell therapy manufacture

Rachel Perret & Kenneth Olsen

An efficient cell selection method is crucial to deliver consistent autologous therapy products when the starting material received is highly variable. Whilst a variety of technologies are being adopted in the industry, there are few GMP-compliant options, and these technologies are often manual, semi-automated, and lack commercial viability. In this article, two experts share insights on obtaining highly purified cells using magnetically active cell selection in a flexible, closed manufacturing system. In addition, transitioning manual cell therapy production to scalable automated processes is discussed.

Cell & Gene Therapy Insights 2022; 8(1), 97–112

DOI: 10.18609/cgti.2022.027

THE CURRENT STATE OF CAR T CELL THERAPY

Since their development in 1989, chimeric antigen receptor (CAR) T cells have evolved greatly, with second- & third-generation CAR T cells incorporating one or two co-stimulatory domains respectively, and fourth-generation CAR T cells including a gene inducer that drives the production of immune effector molecules such as cytokines and chemokines.

CD19 CAR T cell therapy has been shown as an effective treatment for relapsed/refractory diffuse large B cell lymphoma, an aggressive form of non-Hodgkin Lymphoma. The survival probability after treatment with CD19 CAR T cells has been shown to be superior to that of conventional chemotherapy for non-Hodgkin lymphoma [1,2].

There are currently five FDA-licensed CAR T cell therapies on the market, all of which

are second-generation CAR T cell therapies, using either a CD28 or 41BB costimulatory domain. However, none of these CAR T cell therapies is currently licensed in New Zealand.

A NOVEL CD19 CELL THERAPY: FROM CHINA TO NEW ZEALAND

The Malaghan Institute of Medical Research in Wellington, New Zealand has developed a clinical CAR T cell product containing a third-generation CD19 CAR construct using CD28 and TLR2 costimulatory domains (Figure 1). This concept was originally developed and tested by Peng Li and colleagues at the Guangzhou Institutes of Biomedicine and Health (GIBH) in China [3].

Guangzhou Institutes of Biomedicine & Health Phase 1 CAR T cell clinical trial

The first-in-human trial of these third-generation T cells was conducted in China, as a Phase 1 dose escalation study with split-dose infusion, using 5×10^4; 5×10^5; 1×10^6 CD19-CAR-T2 (1928zT2) T cells/kg. The study treated 29 patients, who were suffering from chemotherapy resistant or refractory $CD19^+$ B cell acute lymphocytic leukemia (B-ALL), with extra-medullary disease [4].

Peripheral blood mononuclear cells (PBMCs) were harvested, and CAR T cells produced. Following a lymphodepleting chemotherapy regimen, the CAR T cells were infused in three separate escalating doses over three days. Patients were monitored throughout the study. Data from the first three patients show CAR T cell expansion in the blood after infusion (Figure 2a, b & d). There was a concomitant drop in CD19 expression in the blood, indicating the CAR T cells were eliminating circulating B cells. Cytokine release syndrome (CRS) of grade 2 or 3 was observed in the three patients, as evidenced by the increasing levels of C-reactive protein (CRP) and IL-6 (Figure 2 g, h & i). This was managed with the standard therapy of tocilizumab and steroids.

Complete clinical responses were seen at all three dose levels, including resolution of the extra-medullary disease. This included patient 3, who received the highest dose, and had complete resolution of their disseminated disease [4].

Introducing the TLR2 CAR T cells to the Malaghan Institute of Medical Research clinic

The Malaghan Institute of Medical Research modified the CAR construct to comply with New Zealand regulatory requirements. The fluorescent GFP tag was removed, and the order of the CD3z and TLR2 signaling domains was inverted to resemble other third-generation CAR T cells in clinical trials. The GMP manufacturing process was also adapted to adhere to New Zealand regulatory requirements [5].

The Phase 1 CAR T cell clinical trial currently running in New Zealand (ENABLE) is a dose escalation study using 5×10^4; 1×10^5; 2×10^5; 5×10^5 WZTL-002 (1928T2z) CAR T cells/kg [6]. As of February 2022, 13 patients with relapsed/refractory B cell non-Hodgkin lymphoma have been treated in this study.

Again, PMBCs were harvested and CAR T cells were produced. Patients received lymphodepleting chemotherapy followed by a single dose infusion of CAR T cells. This trial is ongoing, so no patient data is currently available.

TRANSLATION OF MALAGHAN'S OPEN, MANUAL MANUFACTURING PROCESS INTO A FULLY AUTOMATED WORKFLOW

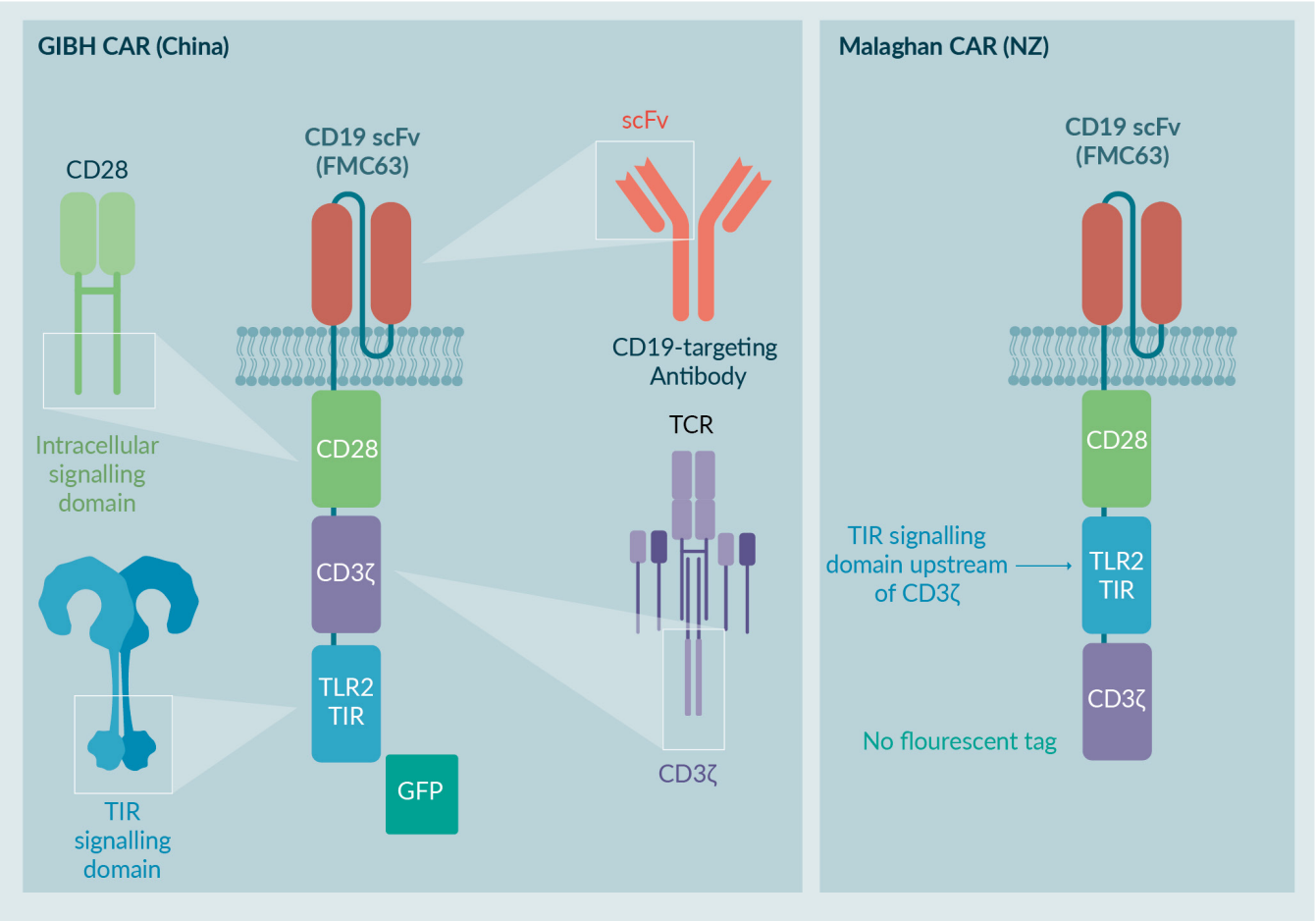
Current manual manufacturing process

The current GMP cell manufacturing process at the Malaghan Institute is fully manual. In a purpose-designed Grade B GMP suite, one or

DOI: 10.18609/cgti.2022.027

FIGURE 1

CAR constructs using a CD19 short chain variable fragment from the antibody FMC63, combined with CD3ζ and the CD28 and toll-like receptor 2 (TLR2) costimulatory domains.



A green fluorescent protein tag allows for detection of the CAR T cells in the original GIBH construct.

two operators can produce one patient's CAR T cell product at a time in what is a labor-intensive process.

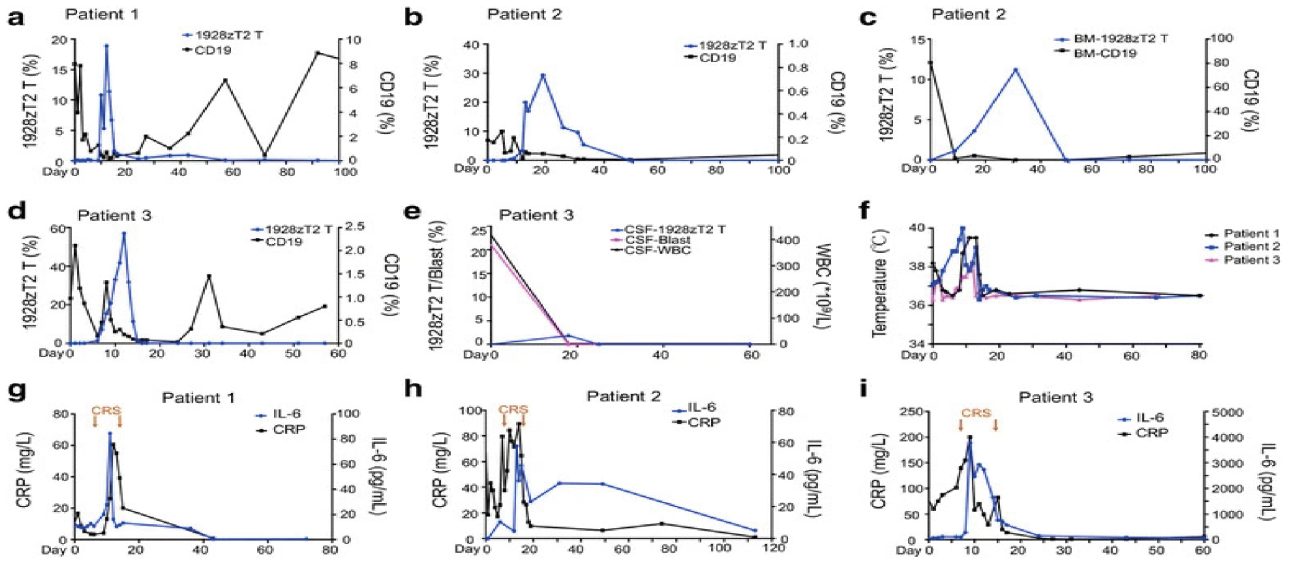
The manual production process begins with frozen PBMCs, which are thawed and rested overnight, before T cells are selected and activated using magnetic beads. The next day, cells are transduced with a lentiviral vector, the remainder of which is removed by manual media exchange on day 3. On day 4, the magnetic beads are removed. There is further media exchange on days 7 and 9, before harvesting and formulating the cells on day 11.

The manual cell manufacturing process was validated using PBMC from 6 healthy donors, whose CAR T cell products expanded well from both fresh and frozen starting material. Three representatives are shown in Figure 3. However, patient cells showed variable expansion with fresh and frozen starting material. Fresh cells generally failed to expand, while frozen cells were more likely to give a moderate to good cell expansion and meet the treatment dose. Representative patient cell expansion profiles are shown in Figure 3.

Several further optimizations were made throughout the process, including alterations to plasticware and integrating cell straining steps to remove any dead or clumping cells. After these optimizations, similar expansion rates in patients to the healthy donors were obtained.

FIGURE 2

Immune monitoring data for the first three patients treated with 1928zT2-GFP CAR T cells.



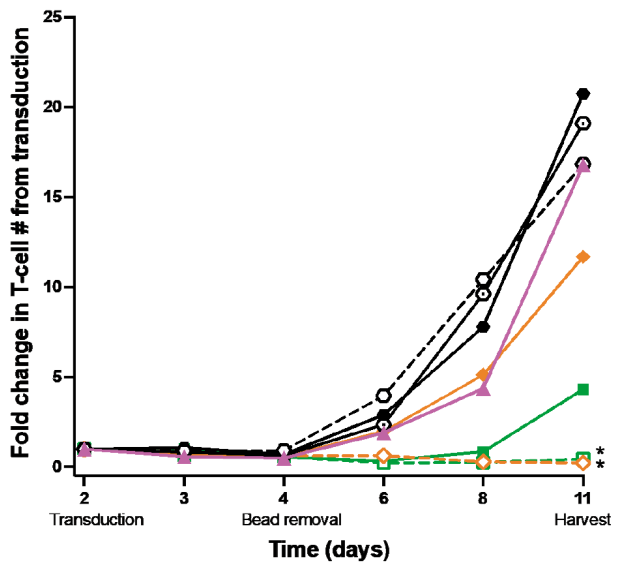
Panels a, b & d show 1928zT2 CAR T-cell expansion and CD19⁺ cell frequency in the blood. Panels g, h & I show IL-6 and CRP levels in the blood. Reproduced with permission from Weng J *et al. J. Hematol. Oncol.* 2018.

Transition to automation to allow for clinical scale-up

Despite this successful optimization, manual cell production has many disadvantages. Only one patient product can be generated at a time due to regulations prohibiting more than one patient's cells being in open culture in a single laboratory. Major operator intervention is required, leading to a time-consuming process. In addition, as the process is in open culture, sterility testing must be performed at the conclusion of the production run.

FIGURE 3

Manual GMP process validation and patient cell manufacturing data.

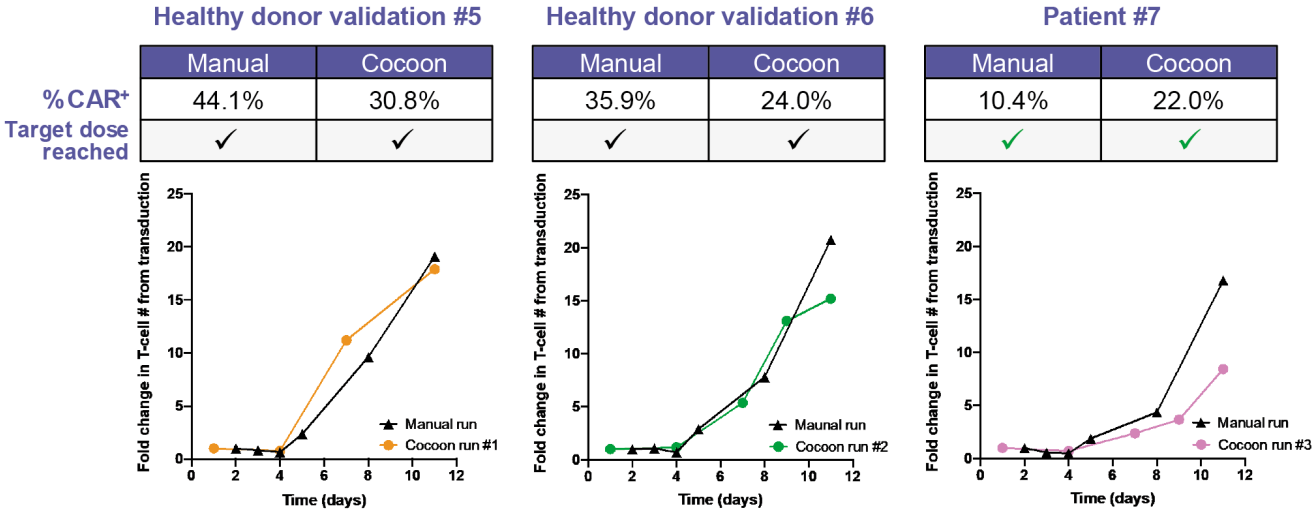


Subject	Run #	Cell source	Dose level	Treatment dose reached
Val_1	1	Fresh	4	✓
Val_5	1	Frozen	4	✓
Val_6	1	Frozen	4	✓
Pt_3	1	Fresh	1	X*
	2	Frozen		✓
Pt_4	1	Fresh	2	X*
	2	Frozen		✓
Pt_7	1	Frozen	3	✓

DOI: 10.18609/cgti.2022.027

FIGURE 4

Comparison of manual and Cocoon® Platform manufacture.



Closed system automation allows for the growth of multiple patient products at once in the same space, subject to regulatory approval by MedSafe. Considerably less operator-intervention is required, and in-process testing of sterility and quality is possible.

The Cocoon® Platform method of automation was chosen because it is compatible with the existing GMP reagents, allows for magnetic separation to be integrated into the process, is a single closed system unit, and has a small footprint allowing for potential scale-out. Lonza's research and development team in Ontario, Canada helped to transfer the manual CAR T cell process to an automated version for use in the Cocoon® Platform.

Although this new process still requires PBMC thaw and rest to be done manually, T cell selection through lentiviral vector transduction, vector removal, bead removal, and media exchange, can all be done in an automated way within the Cocoon® Platform. This requires minimal manual intervention on a few days throughout the process. Formulation after cell harvest is still done manually in the current process, but there are possibilities to automate the PBMC processing and formulation steps in the future.

In terms of CAR T cell expansion, the Cocoon® Platform performs favorably when compared to the manual process (Figure 4). For both healthy donors tested, the current

FIGURE 5

Dynabeads™ CD3/CD28 separation: comparison of the automated Cocoon® Platform to manual control.

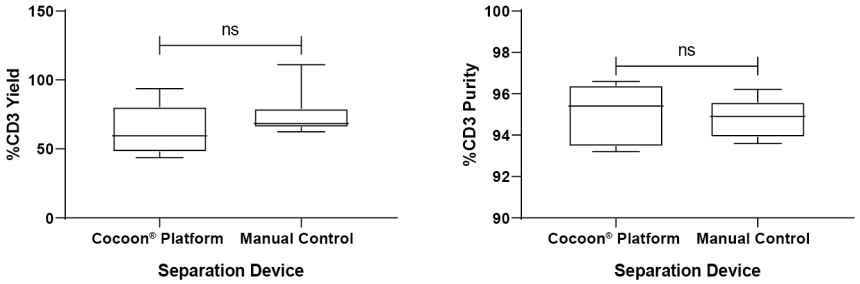
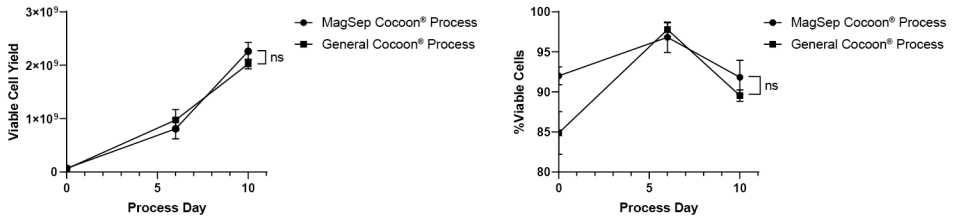


FIGURE 6

Comparisons between the performance of the current Cocoon® Platform and the newly integrated magnetic separation feature.

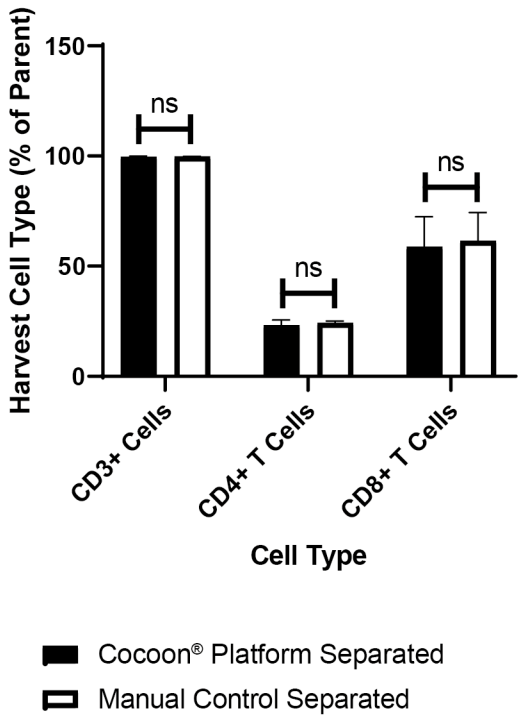


maximum dose level was achieved in both the manual and Cocoon® Platform runs.

Due to minor variability in CAR T cell percentage, the protocol was further tweaked. Successful production of the treatment dose in both the manual and Cocoon® Platform runs was achieved in patient 7, with the CAR T cell transduction efficiency in the Cocoon® Platform being superior to that of the manual process.

FIGURE 7

The CD4 CD8 sub-population ratio for both the new Cocoon® Platform performance and traditional manual control.



Vision for the future

The Malaghan Institute has formed a joint venture with BioOra to co-fund CAR T cell automation in New Zealand. In the short-term, deployment of Cocoon® Platform-produced cells within an expansion cohort of the ENABLE Phase 1 trial is planned, alongside the implementation of in-process quality testing to speed up the product release process.

In the medium-term, priorities include increasing production capacity without increasing the facility footprint, by running several Cocoon® Platforms in the same space.

Long-term plans involve scale-up and scale-out of CAR T cell production by creating a dedicated manufacturing site run by BioOra, allowing for larger scale deployment of CAR T cells to be used commercially or within clinical trials.

THE COCOON® PLATFORM EXPLAINED

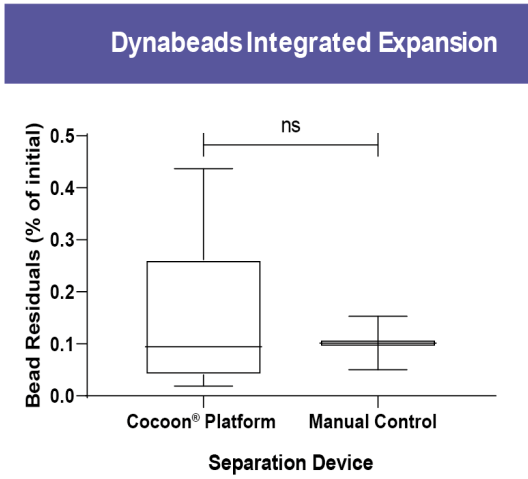
The Cocoon® Platform is a functionally closed automated system designed to reduce touchpoints, thus reducing human error, increasing reliability, improving product quality, and reducing labor and costs. This system can be used in Grade C cleanrooms, which are anticipated to become more prevalent in the coming years.

The inside chamber within the Cocoon® Platform is divided into a top section maintained at 37°C with controlled CO_2 levels, and a bottom section maintained at 4°C

DOI: 10.18609/cgti.2022.027

FIGURE 8

Removal of residual bead efficacy using the Cocoon® Platform and possible treatment doses.



Treatment Doses (Car-T cells/ kg):

01 Dose 1 = 50K/ kg

02 Dose 2 = 100K/ kg

03 Dose 3 = 500K/ kg

04 Dose 4 = 1M/ kg

% Car-T	Dose 1	Dose 2	Dose 3	Dose 4
30.8	136	273	1,360	2,724

Residual Beads/ kg

where fresh media is safely stored. In the center of the top section is a peristaltic pump, which moves the fluid around inside the cassette. Actuators along the lower portion of the top section open and close fluidic pathways within the cassette. Built-in sensors monitor levels of dissolved oxygen and pH, providing feedback during the process.

Integrated magnetic separation

Integrated magnetic separation is a new feature soon to be introduced for the Cocoon® Platform. This will enable the platform to be capable of fully automating the whole process between cell expansion and final formulation, including bead removal.

There have been two major modifications that allow for the integrated magnetic separation. One is on the new magnetic separation cassette, which has a magnetic separation line where the separation occurs. The second is the addition of an internal magnet, which can be toggled 'on' and 'off'. The magnetic separation Cocoon® Platform is suitable for positive or negative selection, as well as bead removal in-cassette. Multiple bead systems have been tested and additional optimization efforts are ongoing.

Beads are captured inside the magnetic separation line. Once captured and separated from the rest of the sample, the beads can either be kept or discarded as needed. This process is entirely automated inside the cassette and Cocoon® Platform.

Magnetic separation – Cocoon® Platform preliminary data

The Cocoon® Platform can achieve upwards of 95% purity following bead separation, with a viability of around 90%. Around 99% of all beads can be removed by the de-beading process. With a seeding count of approximately 60 million cells, 2 billion cells can be produced by day 10 (a 30-fold expansion) whilst maintaining a viability of 90%.

No significant difference is observed in terms of purity of cells separated using the Cocoon® Platform compared to the control, when using CD3/CD28 Dynabeads (Figure 5).

No significant difference can be seen in terms of final cell yield or final viability by day 10 between the Cocoon® Platform and the magnetic separation Cocoon® Platform (Figures 6 & 7).

There is no significant difference between the new Cocoon® Platform and the manual control (Figure 8). The integrated magnet is capable of consistently removing over 99.5% of the initial beads and is not significantly different from manual processes.

From a treatment perspective, even at the highest dose, the Cocoon® Platform can reach 35 times lower bead residuals than the currently used published standard of 96,000 beads/kg [7].

KEY INSIGHTS

The Cocoon® Platform will allow for full automation of CAR T production for clinical scale at the Malaghan Institute in a flexible, closed manufacturing system. The platform's new magnetic separation feature will allow for an efficient, automated bead removal step. Together, these will provide a fully scalable method of producing consistent cellular starting material for autologous cell therapies.

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ASK THE EXPERTS

David McCall, Editor, BioInsights, **speaks to** (from left to right) **Rachel Perret,** Team Leader, CAR T-cell Research Program, Malaghan Institute of Medical Research**, and Kenneth Olsen,** Senior R&D Scientist, Personalized Medicine Business Unit, Lonza.

DOI: 10.18609/cgti.2022.027

Q What advantages did the team see in moving to fully automated systems?

RP: The main preliminary advantage we have seen is an increase in our production capacity, so that we can treat more patients faster. The two main reasons for this are that we can produce multiple CAR T cell products at the same time in our existing facility space, and that we can use our existing staff to be that capacity.

Q Can you expand on your experience with process translation into the Cocoon® Platform and the comparability study?

RP: We are still in the R&D phase at the moment. We are able to obtain pretty comparable results in the Cocoon® Platform with both healthy donor cells and patient cells, although patient cells are more difficult to work with.

We were able to work with the Lonza team to optimize the process as we went along and have found that we can achieve good CAR T cell expansion and good CAR expression in the Cocoon® Platform. The Cocoon® Platform is the logical next step for us to expand our CAR T cell production options at the Malaghan.

Q Does Malaghan have plans to develop additional therapies? Would process development (PD) be done in the Cocoon® Platform, or in another vessel?

RP: We hope to roll out further CAR T cell trials in the future. We are working on different strategies such as dual antigen targeting CAR T cells, and through our collaborations we have options to move into the solid tumor field in the future.

We would do all our early PD manually as it allows for more flexibility at the very small scale in early stages. Then we would transition to the Cocoon® Platform once we have the preliminary process in place.

Q Does Malaghan have plans to manufacture in Grade C or unclassified space at any point?

RP: Not currently. Our regulators require us to work in a Grade A tissue culture hood and a Grade B background for our open cell culture work. As parts of our process are still going to be open (leukapheresis processing and cell harvest), at present we need to remain in a Grade B background.

Q How many runs have you performed with the magnetic separation feature?

KO: We have done lots of partial runs in order to optimize the magnetic separation process. We are building on top of a process that already exists, and we know works quite well.

We have done 13 full runs from beginning to end with the magnetic separation feature, with several more in process currently.

Q How many types of beads have you tested on the Cocoon® Platform?

KO: Currently, most of our efforts on this project have been using Dynabeads. We have begun testing on several other different beads that are on the market, including some nano-scale beads.

Q Is the Cocoon® Platform with magnetic separation ready for sale?

KO: Currently, it is available for presale. If you contact us, we can begin that process.

We expect actual shipments of the Cocoon® Platform with the magnetic separation to be available in Q1 of next year.

Q Will Lonza develop bead solutions for dissolvable nanobeads?

KO: Currently, our strategy is to support a wide range of commercially available bead types, as we want our platform to be as flexible as possible. We do not have any specific plans for supporting dissolvable nanobeads. If this is something that is required for a particular process, we encourage scientists to reach out to us see if we can make this a solution.

Q How do you select T cells after obtaining PBMCs?

RP: We do a buffy coat (Lymphoprep™) separation, so PBMCs are obtained by density gradient. We then isolate the required T cells by magnetic positive selection using CD3/CD28 Dynabeads. This step is performed within the Cocoon® Platform during the automated process.

Q Why did you choose Cocoon® Platform?

RP: We looked at all the different options on the market; there are some other great technologies. The Cocoon® Platform had the advantage of being a small, single, and closed unit which fits well with our existing capacity and plans to scale-up. Also, it is compatible with the type of beads we were using for cell selection and activation.

DOI: 10.18609/cgti.2022.027

Q What were the tweaks to enhance transduction efficiency?

RP: The Cocoon® Platform protocol was adapted from our fully optimized manual process, so it ran a lot more smoothly than the initial manual attempts, because we were far more advanced in our process at this stage. However, we had to do many tweaks, including to cell concentration, to the processing of cells, the way the lentivirus is added to the cells, as well as some of those little tweaks you get a feel for when working with your hands in the lab.

I cannot comment too much on the actual modifications that were made in the Cocoon® Platform because they were done by the developers in Canada.

Q Why do frozen cells expand better than fresh cells?

RP: We are not 100% sure about the reason behind this. We did not expect it, especially based on what happens with healthy donor cells.

Lymphoma patients have a very different leukocyte and neutrophil make-up to a healthy person, both due to the cancer and the pre-treatments they go through. It is most likely that an inhibitory or suppressive population is being lost during the freeze-thaw process, like granulocytes or monocytes, so we are getting a cleaner lymphocyte preparation.

Q Are the residual beads after removal still functional, or are they decomposed so that just non-functional magnetic particles are left?

RP: The beads are antibody-coated, which are not long-term stable conjugations, so the antibodies will detach from the beads and the beads detach from the cells. We can see under a microscope that the beads do not remain connected to the T cells. They are magnetic particles that we do not want to transfer to the patients in large numbers.

Q Why do you need to remove the beads?

KO: Our data shows that expansion increases following bead removal whether at day 3 or day 7. The mechanism for this is an ongoing investigation. The best cell yield seems to be when removing beads either day 3 or 4 but can be delayed if it's better for your specific process or needs.

Q Can the Cocoon® Platform perform a de-beading step for the Dynabeads process?

KO: As Rachel mentioned, the antibodies are conjugated to the beads, so unfortunately there is no way to remove the beads from the cells at the beginning of the process. We need to wait until the beads naturally detach from the cells. Once the beads are removed from the system, we can proceed with the rest of the run without having beads inside the sample.

Q How long does the automated cell separation process take?

KO: The magnetic separation process takes approximately 12 minutes. We pass our sample through the magnetic separation line multiple times to increase the yield. The 12-minute run time is the total time it takes for the entire sample to pass through that magnetic separation line and collect the beads that have been pulled out, repeated multiple times.

Q Does the Cocoon® Platform do the bead mixing or does the user pre-mix cells?

KO: Currently, the process does involve mixing the beads with the cells outside of the Cocoon® Platform, then loading them into the cassette where the magnetic separation occurs. We are currently developing processes to try to automate this entire process.

Q Is the Cocoon® Platform process including magnetic separation available for other cell types such as NK or DC?

KO: Currently, the process is not optimized for those types of cells. The Cocoon® Platform itself is capable of working with many different cell types. If there is a specific need for a different cell type, our team can figure out if those specific cell types and processes have already been looked at or it seems like a viable solution.

Q Is the Cocoon® Platform an open development platform?

KO: While the end users will be capable of developing the processes, we have teams that can work with scientists to ensure that various steps are optimized and that the process is going to be as successful as possible.

Q Is the automation package 21CFR part 11 compliant?

KO: Yes, it is. That is one of the main features that we have made sure to have in the automated process, so that it can be used where needed in GMP spaces.

DOI: 10.18609/cgti.2022.027

Q What is the multiplicity of infection (MOI) used?

RP: I believe that is an MOI of 4.

Q Your data show 99% bead removal. Can you achieve that after day 3?

KO: The 99%, or greater than 99%, data presented is bead removal at the very end of the process, just before final harvest on the last day of the run. We do an additional bead removal step, with which we can achieve the 99%.

Q What is the maximum input of cell number, and at what bead ratio?

KO: Currently the highest number of cells that we have used in the system is 450 million total $CD3^+$ cells, at a bead ratio of 2:1. Our system is quite capable of handling roughly around 1 billion cells.

Q What are the minimum volumes on the Cocoon® Platform?

KO: During the beginning steps for introduction of cells into the cassette, the minimum number of cells that we recommend is 30 million cells, seeded, typically in 30ml of solution.

RP: Our process manual process has a starting number on the lower end of the scale. So, in the Cocoon® Platform we also start with the low number of cells at a concentration of about 1 million cells/ml. Due to media recirculation and continuous monitoring of oxygen content and pH levels in the Cocoon® Platform, we do not have to constantly count and re-seed cells at particular concentrations throughout the process. They can expand well with a constant flow of media circulating throughout the proliferation chamber.

Q Can you comment on the negative versus positive selection processes on the Cocoon® Platform?

KO: We have done a few preliminary studies looking at the differences between negative and positive selection – they both show good results. It really depends on your process needs. The negative selection can be more costly, but if a process requires that the targeted cells are not bound to magnetic beads then that might be the only solution. Our system is capable of managing that as well.

Q How many cells are you able to harvest at the end of your Cocoon® Platform?

RP: It depends on the patient starting material. We have had final products ranging from anything between 4×10^8 and 2×10^9 cells at the end of the process.

Q Is there a specific cassette for immunoselection?

KO: We have a specific cassette for magnetic separation. It is not available on the previous cassettes that we have been using. However, the new cassette that we are developing in connection with this magnetic separation capability will become the standard cassette moving forward. Whether or not the feature is needed, it will be available.

Q Are the magnets in the Cocoon® Platform developed to also work with smaller magnetic beads?

KO: Currently, most of our work has been done on the larger Dynabeads. However, we are beginning some investigative work on the smaller range. We are working on optimizing steps so that we can support both types of beads within the same system.

Q Could the Cocoon® Platform be compatible with transfection instead of transduction?

KO: I have not been involved in any of those studies currently, although we are looking into certain technologies that could be integrated with the Cocoon® Platform. We are going to expand the capabilities in the future, for more than just transduction.

BIOGRAPHIES

Rachel Perret

Team Leader, CAR T-cell Research Program, Malaghan Institute of Medical Research

Dr. Rachel Perret is a Senior Cancer Research Fellow and Team Leader of the CAR T-cell Research Programme at New Zealand's Malaghan Institute of Medical Research. She studied Microbiology at the University of Otago, NZ, before undertaking her PhD in Immunology with Professor Franca Ronchese at the Malaghan Institute. Rachel spent several years overseas, first at the Lausanne Branch of the Ludwig Institute for Cancer Research, and then at the Fred Hutchinson Cancer Research Center, and returned to the Malaghan Institute in January 2020. Her research focus lies in investigating chimeric antigen receptor (CAR) T-cell signalling and function, in order to design even better, safer, and more broadly applicable CAR T-cell therapies for cancer.

DOI: 10.18609/cgti.2022.027

Kenneth Olsen

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Dr. Kenneth Olsen received his BSc in Biomedical Sciences, and his Masters in Biology and Vision Science from the University of Waterloo. He received his PhD from the Institute of Medical Sciences at the University of Toronto where he developed a novel device for generating biomechanical compression as a cellular model for glaucoma. After a postdoctoral research associate position at the University of Utah, and a Postdoctoral Fellow at the Canadian Centre for Alternative to Animal Methods at the University of Windsor, Dr. Olsen joined the Lonza Personalized Medicine R&D team as a Senior Scientist in July 2021.

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

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

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

ARTICLE & COPYRIGHT INFORMATION

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

Webinar recorded: *Nov 23 2021;* ***Revised manuscript received:*** *Feb 4 2022;* ***Publication date:*** *Feb 10 2022.*

Lonza
Cell & Gene

The Cocoon® Platform

Brought to you by Lonza, leveraging 20 years of cell and gene therapy manufacturing experience.

The Cocoon® Platform is a closed and automated solution with magnetic selection capability for patient-scale cell therapy manufacturing.

Using flexible programming, and in-process monitoring, the Cocoon® Platform provides increased control over your manufacturing process.

Superior scalability

The Cocoon® Tree arrays Cocoons vertically with a 1m² footprint providing superior scalability and minimizing clean room space requirements.

Prototype in development. Form seen here may not be exact representation of final product.

What does this mean for you?
– Improved product quality
– Superior scalability
– Lower costs

Through the Cocoon® Platform's single-use, highly customizable cassette we can tailor the cassette and programming to your process rather than requiring you to fit your process to the hardware. This provides flexibility when migrating to a closed, automated system.

The Cocoon® Platform is your solution for cell therapy manufacturing from pre-clinical to commercial-scale; allowing you to move quickly and efficiently, with your established process through each phase.

Whether searching for a manufacturing partner, independently performing development and/or manufacturing, or somewhere in between, Lonza and the Cocoon® Platform offer you the technology, knowhow, and partner for success.

Website: **lonza.com/cocoon** Email: **cocoon@lonza.com**

CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Developing stem cell therapies: expansion strategies and lessons learned

Catherine Siler, Shirley Mei & Josée Champagne

The field of mesenchymal stromal cell therapy for critically ill patients has been evolving rapidly. In this article, Corning Field Applications Scientist Catherine Siler will present tools and process design strategies for achieving consistent and efficient expansion of therapeutic cells, including scale-up and scale-out options and closed system designs. Then, Ottawa Hospital Research Institute scientists Shirley Mei and Josée Champagne will put the technology in context by discussing how their team translated a mesenchymal stromal therapy manufacturing process from the preclinical laboratory to a GMP setting.

Cell & Gene Therapy Insights 2022; 8(1), 113–126

DOI: 10.18609/cgti.2022.024

EXPANSION OF MESENCHYMAL STROMAL CELLS: TECHNICAL CHALLENGES & SOLUTIONS

Catherine Siler

Mesenchymal stem/stromal cells (MSCs) present an attractive target for cell therapies for several reasons, not least that they can be derived from multiple tissues, including adult adipose tissue and bone marrow, and newborn umbilical cord (Figure 1).

Challenges in MSC culture

As seen in (Figure 1), MSCs can be derived from a variety of sources, some more readily available than others. MSCs derived from different sources may behave differently in culture and, like any patient-derived material, there may also be differences between donors. All of this must be considered when designing a culture process.

It is also important to consider the medium used. Traditionally, MSC culture medium would include either a serum component or, for

those looking to stay xeno-free, human platelet lysate. Although both of those support robust culture, applications that are geared towards human therapeutics might ultimately steer away from any type of serum component. For those in a clinically oriented setting, there are several types of media available that are chemically defined, and therapeutic developers must test and choose the variety that best supports their culture goals.

Although MSCs grow well in adherent culture, it is important that they retain their phenotype and potency while in culture. For new processes, researchers may want to assess identity using some of the markers previously mentioned.

Perhaps the greatest challenge that MSCs present is the significant number of cells required to achieve therapeutic effects *in vivo* – up to 2 million cells per kilogram of body weight, adding up to hundreds of millions of cells for an adult patient. This requires a large number of vessels and could put pressure on the staffing or space in a facility. Hence, it's critical to have a robust scale-up plan.

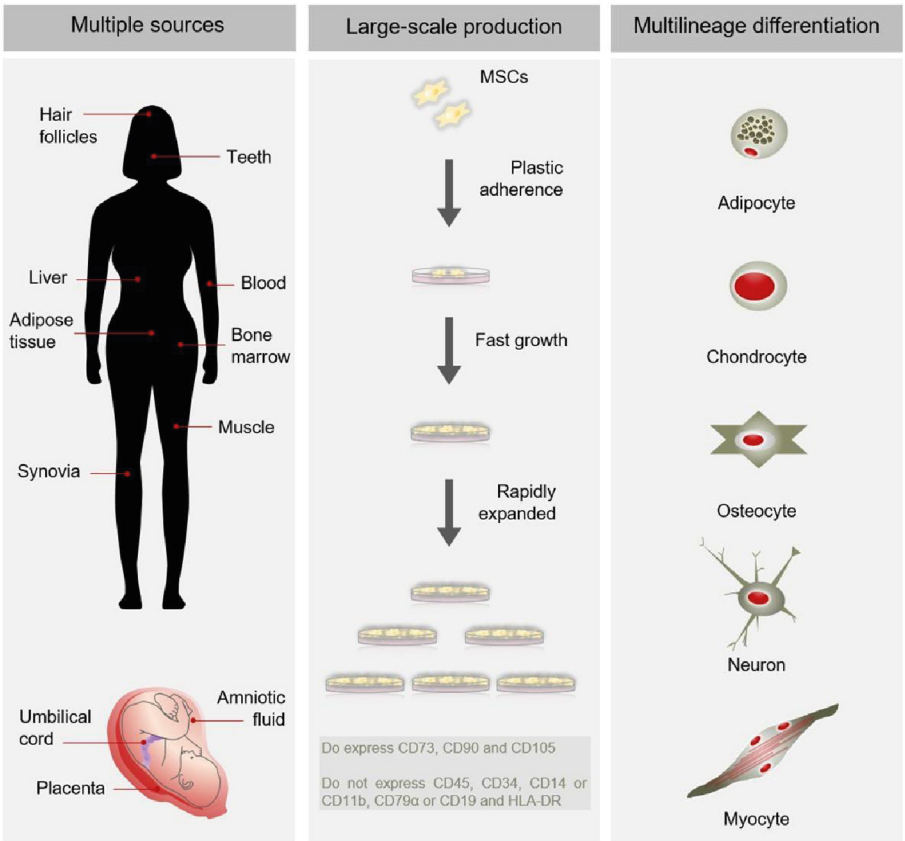
Manufacturing at scale

The first consideration in developing a seed train is what will keep the cells growing and functioning optimally. Some users may want to achieve a greater confluence before passaging, while others might want to prevent them from getting to a certain percentage confluence.

With that in mind, the starting material or cell bank is an important factor. MSCs can become senescent over time, and it is important to harvest their capabilities before their growth slows down, so population doublings are an

FIGURE 1

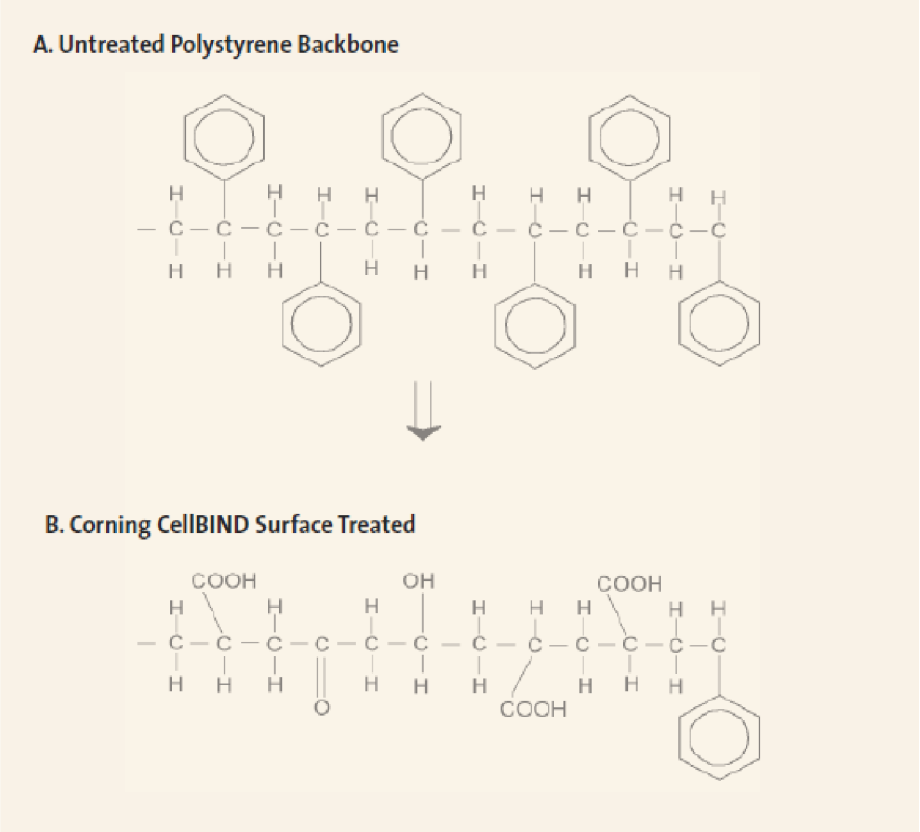
MSC overview.



DOI: 10.18609/cgti.2022.024

FIGURE 2

Corning CellBIND surface facilitates cell attachment.



important factor. It is also valuable to consider how many passages the cells will experience in the scale-up process because this has implications for both cell health and process efficiency.

Whatever the next step in vessel size or type, it is important to consider the consistency of the culture environment, including the ratio of media to surface area and the treatment of the surface the cells are growing on. Because the chemical structure of an untreated polystyrene is oily and very hydrophobic, many Corning® vessels feature the CellBIND® surface. The CellBIND treatment makes polystyrene more hydrophilic by the addition of oxygen-containing groups like hydroxyls, carboxyls, and carbonyls (Figure 2).

Scale-up or scale-out?

A final consideration for the seed train is the space in which a given process will occur, and therefore whether to scale out or scale up. Scale-out models add vessels of a similar size or type, whereas scale-up models introduce a larger vessel, which may be of a different type (Figure 3).

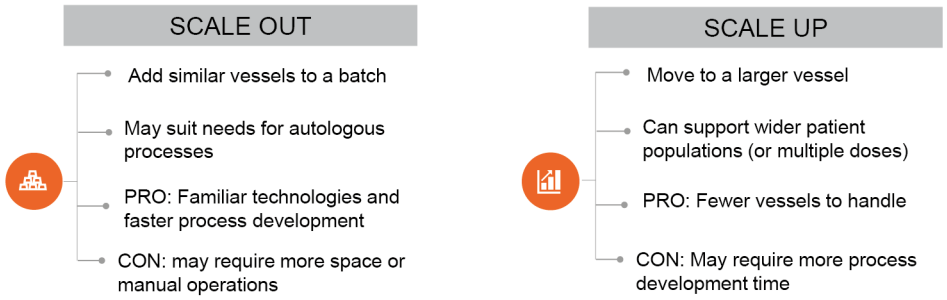
Technology for scale

Corning has a wide range of cell culture vessels for scale up or scale out, which can be seen in (Figure 4).

To demonstrate the advantages of the technology, HYPER vessels were employed in a seed train, which was designed with the goals of rapid expansion, a consistent cell culture surface, and minimal scale-up steps. Umbilical cord-derived MSCs were taken from thaw and cultured in a T-175 flask until they reached 90% confluence. After passaging, the cells were expanded into a HYPERFlask at a density of 3,000 cells

FIGURE 3

Pros and cons of scale up versus scale out.



per cm^2 and after 5 days were passaged into a HYPERStack 36-layer vessel, with 18,000 cm^2 of growth area.

The HYPERStack is a closed system vessel that allows users to transfer liquids more safely and can ultimately be used in GMP production. For clinical material, risk mitigation is critical, and closed systems play an important role in that process.

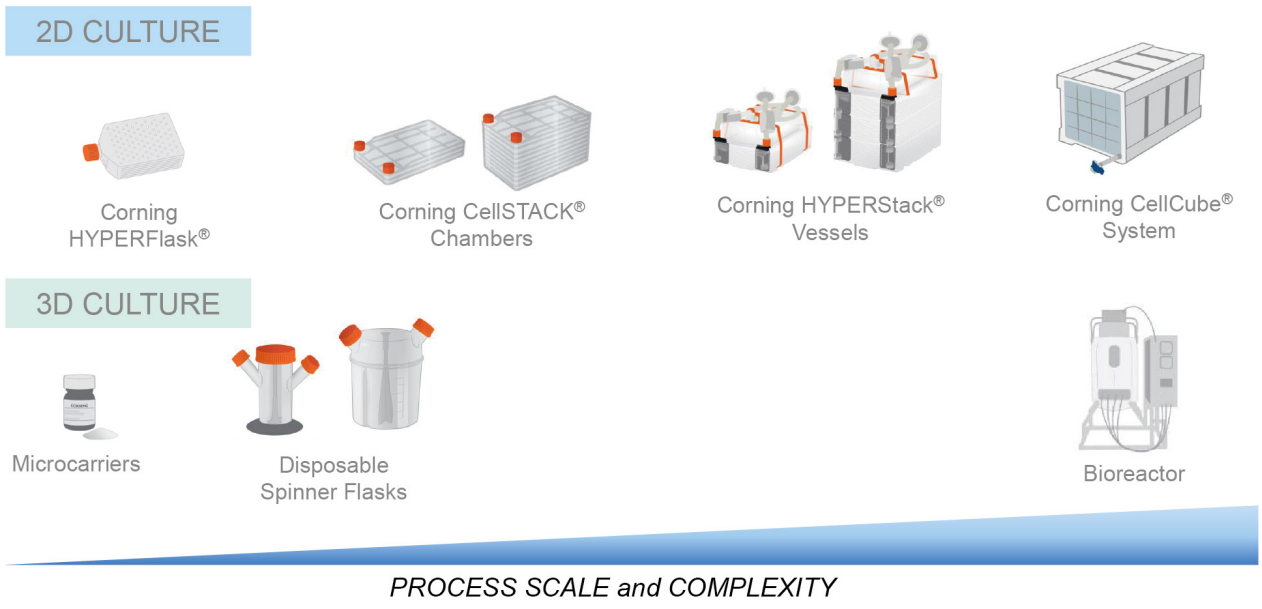
A surface area comparison can be seen in Table 1.

Over 60,000 cells per cm^2 were recovered after five days of culture in the HYPERStack vessel. The average of three studies results in a total MSC yield of over 8.7 x 10^8 cells per HYPERStack-36, with consistent viability at 90%. Of note, the marker expression at the end of the process was consistent with the starting material and fits the criteria put forth by the International Society for Cellular Gene Therapy, with greater than 95% expression of CD105, 73, and 90, and no expression of typical hematopoietic markers.

Moving into larger scales of 10 billion cells or more, HYPERStack can be used in larger scale-out models such as those using manifolds and automated manipulators. At manufacturing scale, Corning offers

FIGURE 4

Cell culture vessels from Corning.



DOI: 10.18609/cgti.2022.024

TABLE 1

Surface area comparison for different vessel types

Vessel	Surface area	Equivalently sized vessel	Increased surface area
Corning® HYPERflask®	1720 cm^2	T-175	10×
Corning® HYPERstack® -36	18,000 cm^2	Corning CellSTACK® -10	3×

bioreactor-related platforms such as microcarriers and the CellCube® system (Figure 5).

CLINICAL PERSPECTIVES: DEVELOPING A CELL THERAPY FOR SEPTIC SHOCK

Shirley Mei & Josée Champagne

Septic shock is a devastating illness. It is characterized by a highly dysregulated immune response, with cardiovascular collapse and organ failure. Despite early identification, aggressive resuscitation, and administration of antibiotics, patients still suffer a mortality rate of 20 – 40%. Those fortunate enough to survive face long-term morbidity associated with physical, cognitive, and emotional dysfunction.

From a healthcare system perspective, sepsis costs more than 4 billion dollars per year to treat and, despite decades of research, no targeted therapeutic agent for septic shock has improved clinical outcomes. Given the significant preclinical evidence, MSCs are believed to represent an exciting potential therapeutic option for this patient population.

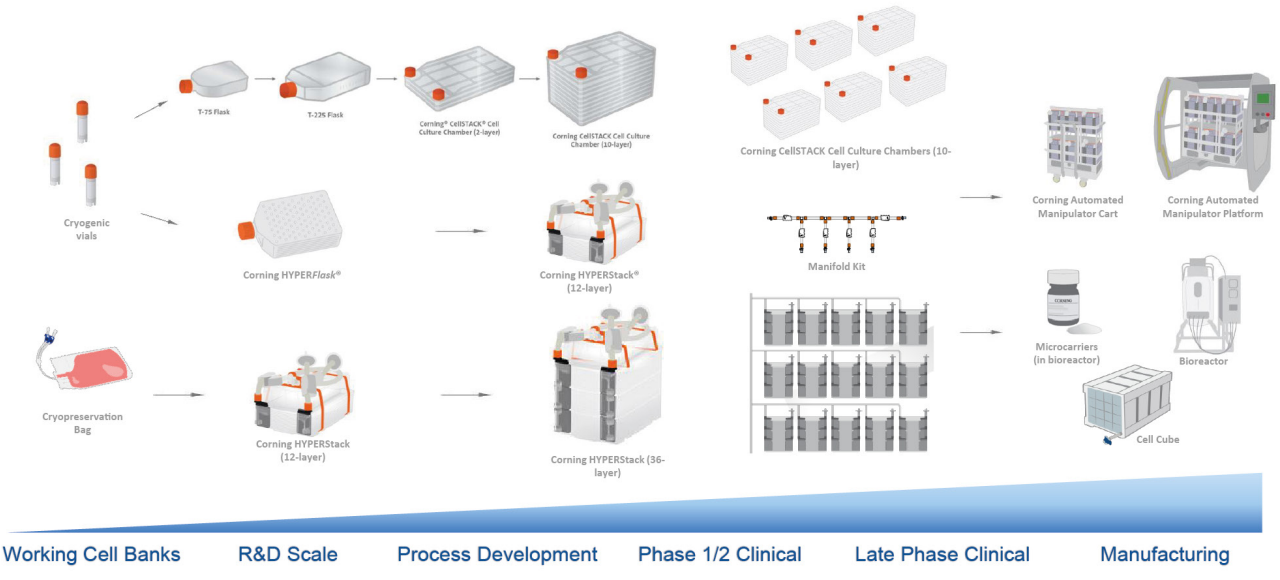
In contrast to chronic diseases, acute and severe conditions such as septic shock require rapid intervention after disease is identified and this intervention must exert a therapeutic effect within hours of administration. Here, we discuss our progress in developing a MSC therapy for septic shock and how our manufacturing process evolved to meet the need for an off-the-shelf product.

Phase 1 trial: CISS1

After numerous promising preclinical studies by our own and other groups, the Regenerative Medicine Program at Ottawa Hospital Research Institute (OHRI) took on the

FIGURE 5

Expansion processes considerations for scaling up and scaling out mesenchymal stromal cells.



challenge of developing a MSC product for testing in a septic shock clinical trial. Our Phase 1 trial, Cellular Immunotherapy for Septic Shock (CISS1), led by Dr Lauralyn McIntyre, was the first in the world to evaluate MSCs in septic shock patients.

The Phase 1, open-label, single-center dose-escalation trial used freshly cultured, allogeneic bone marrow-derived MSCs. The product was delivered as a single IV fusion to patients with septic shock, to examine the safety and tolerability of MSCs in this population setting.

Our secondary outcomes included the serial collection of biomarkers of inflammation over time and feasibility related to the operations and conduct of an MSC trial in this patient population.

Manufacturing

During the manufacturing development process, one of the biggest challenges initially for the team was on how to scale up from the small number of cells typically used in animal experiments to the large number of cells required to dose a patient.

We adopted a simple strategy, using a 10-layer Corning HYPERFlask. HYPERFlask vessels have around ten times the surface area of a similarly sized T175 flask and we found it can produce a proportional increase of cell yield. In addition, the similar size of a HYPERFlask to a standard T175 flask made the technology transfer process relatively simple when scaling up to the manufacturing facility.

(Figure 6) shows how the MSC product was manufactured for the Phase 1 clinical trial.

A master cell bank (MCB) was derived by seeding bone marrow aspirate from a healthy donor and the cells were further expanded to derive vials of MCB. The release specification for the MCB used in the CISS1 trial included but were not limited to cell counts, viability by hemocytometer count, surface marker expression, endotoxin, and sterility tests.

These MCB vials were subsequently used to seed a new set of HYPERFlask vessels weekly to allow a continuous supply of fresh cells. This strategy meant that the manufacturing facility need to harvest the cells on demand and deliver them to the ICU within a short time of an eligible patient being enrolled.

Given the very short time window to have the product ready to treat a patient, the release specification for the fresh MSC product used in the Phase 1 CISS trial included e a visual assessment for the appearance of MSC morphology, Trypan Blue count to confirm cell dose and concentration, and endotoxin level checks.

Trial design and results

Eligible septic patients were enrolled within 24 hours of first admission to the ICU. A control group of 21 participants who met the same eligibility criteria but did not receive MSCs was prospectively enrolled in advance of initiating the MSC interventional arm of the trial, to characterize the incidents of expected adverse events and serve as a comparator for the interventional group.

In the MSC interventional group, there were three separate MSC dose cohorts, with three participants per cohort, who received doses of 0.3, 1, or 3 x 10^6 cells per kg, to a maximum of 300 million MSCs.

Serial plasma samples were collected at various time points. Participants were monitored for MSC transfusion-associated events and serious unexpected adverse events for 1-year post-MSC transfusion. An independent data safety monitoring board (DSMB) reviewed the data following each cohort.

In conclusion, the Phase 1 trial determined that MSC doses of up to 3 million cells per kilogram appear safe, and that it was feasible to use MSCs in adult septic shock patients. We are now moving on to a much larger Phase 2, pan-Canadian, randomized, placebo-controlled trial [1].

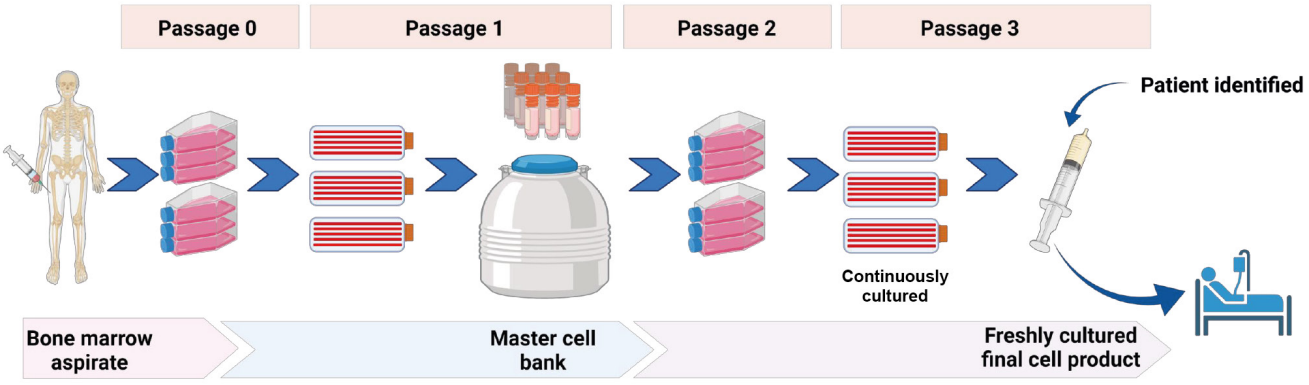
Phase 2 trial: CISS2

The CISS1 trial showed that delivering freshly cultured cell products to septic

DOI: 10.18609/cgti.2022.024

FIGURE 6

Fresh MSC product manufacturing for Phase 1 CISS clinical trial.



Created by Yuan Tan via BioRender.com.

patients, while possible, is difficult and can be costly. In particular, the use of fresh cell product could present challenges for hospitals without an established cell manufacturing facility within or nearby. The goal of our teams is to have the product ready to be administered at very short notice and it was clear that an off-the-shelf MSC product was needed to achieve that. Ahead of the Phase 2 trial, we started working on developing a process to cryopreserve the final cell product, so that it can be stored before administration.

Manufacturing

Further studies were required to confirm the comparability of fresh versus frozen cells, which will be used for the larger Phase 2 trial [2]. We compared cell recovery, viability, cell identity, and potency *in vitro*. Both exhibited similar surface marker profiles, viabilities, and *in vitro* potency. We then compared the potency of fresh and frozen products in an animal model of sepsis and found that both products were equally effective in recovering and even improving the phagocytic ability of the peritoneal lavage cells from septic mice (Figure 7).

Taken together, these studies showed that the frozen product maintained good cell viability after cryopreservation and thawing, with similar functionality and potency to freshly harvested MSC.

Animal toxicology studies with the frozen MSC product also demonstrated comparability with fresh cells and showed no MSC-infusion-associated changes, no changes in kidney and liver function clinical chemistry parameters, no changes in body weight, and no changes in the histopathology of organ weight. Biodistribution studies found no trace of human genomic sequence at 60 days after the product infusion.

Trial design

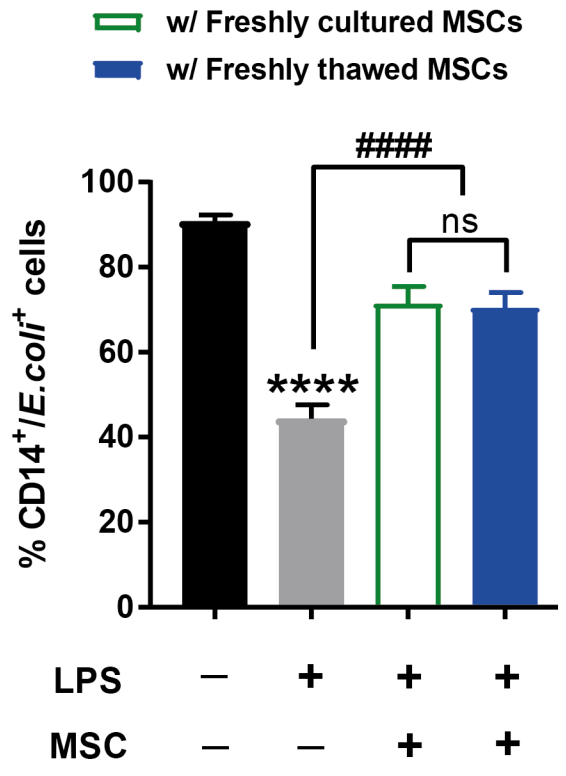
The primary aim of the Phase 2 randomized, controlled trial (RCT) is to determine whether a single IV infusion of 300 million cryopreserved MSCs (the highest MSC dose from our CISS Phase 1 trial) reduces organ failures compared with placebo, in 122 patients across ten academic centers in Canada.

The frequency of adverse events and serious adverse events will be reported, and blood will be drawn for inflammation markers at baseline 12, 24, 72 hours, and 7 days post-infusion. The DSMB will be convened during the trial to review adverse events and/or clinical endpoints, and *a priori* adverse events.

Recently, the clinical trial protocol has been updated and reviewed by our executive

FIGURE 7

Fresh and frozen MSC potency *in vivo*.



n = 3~6 experiments
One way ANOVA
post hoc Bonferroni's test. ####P < 0.0001

Mice were randomized to CLP (cecal-ligation-and-puncture, sick animal) procedure to induce sepsis or sham operation (normal animal). Either fresh or frozen MSC were administered to septic mice at 6 hours after. At 24 hours, peritoneal CD11 expressing cells were recovered from each animal, and tested for their ability to phagocytose bacteria. Black: Healthy (sham animal), untreated. Gray: Septic, untreated. White: Septic, treated with fresh MSCs. Blue: Septic, treated with frozen MSCs. Reproduced from [2].

committee, composed of multiple investigators across Canada, with the hope of submitting to Health Canada in January 2022, and we anticipate enrolling our first participant in Spring 2022.

Lessons learned from the translational process

When translating a promising lab research product from animal studies into human trials, think early and carefully about the product you want to use for your trial. Areas for consideration include:

- Logistics, including cold chain transportation, storage and transport.
- Type of patient population (acute vs chronic).
- Is the production process scalable beyond Phase 1 and possible to commercialize?
- Technology transfer: is your process transferable to a CDMO manufacturing facility?
- Cost of Goods: identify cost-saving opportunities during manufacturing process development, such as reducing media reagent, or developing a process with higher cell yield.

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DOI: 10.18609/cgti.2022.024

ASK THE EXPERTS



Charlotte Barker, Editor, BioInsights, **speaks to** (from left to right) **Catherine Siler,** Field Applications Scientist, Corning Life Sciences, **Josée Champagne,** Senior Research Associate, Ottawa Hospital Research Institute, **and Shirley Mei,** Investigator, Ottawa Hospital Research Institute.

Q What do you see as the main advantages of using MSCs in cell therapy?

SM: The main advantage is that MSCs have been considered almost as a type of universal donor cell. Many cell therapies need the donor and recipient to be matched, but numerous clinical trials in various patient groups have not reported evidence of rejection with MSC cell therapy.

CS: The thing that fascinates me about MSCs is that many of the sources are fairly accessible. For example, adipose tissue is removed from people undergoing liposuction every day, and it is great to think that it can be used to create therapies when it would otherwise be discarded.

Then there are the various applications that it can be geared towards – areas like graft versus host disease, where there are few existing treatment options.

Q What are some of the safety considerations when designing an early-phase trial?

JC: We started by carrying out systematic reviews of preclinical and clinical studies to help identify (a) what type of *a priori* adverse events we could build into the trial protocol and (b) lessons learned from groups that have previously done similar work.

When designing the protocol, Dr McIntyre, along with the Canadian Critical Care Trials Group (CCCTG), were particularly mindful that adverse events in ICU-admitted septic shock patients occur daily; and it is very difficult to attribute causality. Instead, the protocol was designed to home in on (and define) what types of adverse events would be reportable. Furthermore, we also included stopping rules around the time of infusion that would, if required, pause recruitment until the data safety monitoring board (DSMB) had a chance to review. The protocol was also designed to allow for a DSMB review after each dose cohort before proceeding to the next dose. Prior to submitting the protocol for Health Canada review, we engaged the regulator early to ensure that our approach to safety was acceptable to them, especially for the first-in-human trial.

Q How do I translate my research workflow into a GMP-grade manufacturing process? What would be your key tips?

CS: One of the biggest transitions is the implementation of closed systems. Whether it's something that you plan to continue doing in-house or outsource to a CDMO, implementing some type of closed system is always part of the conversation. Every process and every facility will probably have slightly different preferences in terms of what they want that closed system to look like. There's really no one-size-fits-all, so we spend a lot of our time talking to customers to help them tailor a process to suit their goals.

Q What are the typical challenges and gaps that emerge when you're having those detailed conversations?

CS: What works for you at the bench scale might not work for you once you get to manufacturing. You can implement a closed system at the bench scale, but those steps might not make sense at manufacturing scale, in terms of the facility or the employee resources, so it's important to plan ahead.

In addition, we have a lot of discussions around making sure that everything works together. It's not unusual that some components come from one vendor, some come from another, and all those pieces need to sit together.

Q What trends are you seeing in MSC research at the moment?

CS: One thing I've seen recently is that people are looking not only at MSC products, but MSC-derived products, for example, extracellular vesicles, which could potentially raise fewer immune concerns. I've definitely had a lot of conversations recently about acellular therapies.

Q How many passages of the master cell bank post-thaw can the process accommodate?

DOI: 10.18609/cgti.2022.024

SM: It depends on the type of MSC (donor source, method of derivation) and how many passages you have processed before you bank it into the master cell bank. In our case, we don't see much senescence in our MSCs until well beyond passage 7, so we keep our passages to 2 or 3, and the population doubling number relatively low. Ultimately, it's up to individual researchers to understand your product.

Q How steep is the learning curve associated with a different scale-up technology?

CS: Moving from a smaller to a larger vessel in the same footprint, for example moving from T175s to CellSTACK®, is a quick way to get up to scale. Moving from an open to a closed system, like HYPERStack, that may be unfamiliar to users, will have a steeper learning curve. The steepest learning curve and the most process development time comes when you are implementing a bioreactor. For example, getting the cells to stick to a microcarrier, determining agitation conditions, and learning to work with whatever your chosen bioreactor platform is. The more advanced the technology, the more process development time is required but the benefits can also be significant.

Q How are MSCs extracted from donors safely, and how do you ensure the cells are healthy?

SM: As we're doing allogeneic cell therapy, our cells are derived from bone marrow from healthy volunteer donors, not from patients themselves. We could give a whole seminar on how we pick our donors given the rigorous donor screening requirement – there is adventitious agent testing and a detailed review of the donor's health history before we determine that they are suitable donors. We also carry out extensive testing of the master cell bank itself. The health of cells isolated can be determined not only by viability assessment but also through their potency and cell population doubling time.

Q Is there an active gas control on the HYPERStack or is it a passive diffusion through the vent filters on the top?

CS: The vent on the HYPERStack is there to facilitate liquid movement during emptying and filling. The tube leading to that vent filter is closed during culture. Otherwise, all of the gas exchange occurs passively through the sides of the vessel. No special gas setup is required – just a typical CO_2 incubator.

Q What is the maximum amount of time that MSCs can be kept frozen and remain viable once thawed?

SM: Again, this is very product-specific, because cells may behave differently depending on tissue source and isolation method. You need to have a matrix of parameters to test your cells to know whether they are performing the same as fresh cells and confirm that you still feel comfortable using them.

For our cell product, we have stability data up to a year and for our master cell bank, we have 5+ years of stability data.

Q Which product performs better, the HYPERStack or bioreactors?

CS: Both will give you a lot of cells, so the decision really depends on your criteria for success, your critical quality attributes, and your process parameters.

If you determine that certain process parameters like the pH or concentration of specific metabolites must be very exact, a bioreactor gives you a method to control those parameters. But other factors, like the cost of goods, capabilities of your facility, and staffing, all have to be factored in. It really is very dependent on the user and what is important to them.

BIOGRAPHIES

Catherine Siler

Field Applications Scientist, Corning Life Sciences

Catherine Siler, PhD, is an accomplished Field Application Scientist for Corning Life Sciences, with a PhD in Biology from Johns Hopkins University. Siler enables scientists and researchers in the life science industry to overcome challenges with cell culture and scale-up for clinical manufacturing of advanced therapies, and utilizes her research and teaching experience to drive the adoption of an industry-leading global product portfolio of innovative single-use consumables for research, process development, and bioprocessing applications.

Josée Champagne

Senior Research Associate, Ottawa Hospital Research Institute

Josee Champagne has 20+ years of experience in both investigator-led and industry-sponsored clinical research. As the International and Regulatory Coordinator for the investigator-led, Phase 3 international Folic Acid Clinical Trial (FACT) she oversaw all aspects of the study, including participant recruitment, trial implementation, drug manufacturing, and importation, and closeout across 96 clinical sites in five different countries. Since 2016, she has been managing the Cellular Immunotherapy in Septic Shock (CISS) Program and has gained considerable expertise interpreting federal regulatory requirements for emerging biotech products, including cell and gene therapies.

Shirley Mei

Investigator, Ottawa Hospital Research Institute

Shirley Mei, PhD, MSc, has 18 years of experience in the nonclinical development and translation of MSC therapy into the clinic for critical illnesses. As a translational scientist, she has been working with ICU clinicians to conduct the worlds' first-in-human clinical trial

DOI: 10.18609/cgti.2022.024

for septic shock patients with allogeneic MSC derived from bone marrow (NCT02421484), in addition to several ongoing cell-based gene therapy trials for critically ill or pulmonary patients. With a focus on bringing discoveries from the bench to the bedside, her current research includes studying how MSCs exert their immunomodulatory effect on immune cells using in vitro culture systems, preclinical disease models, and clinical samples; and developing novel molecular strategies to enhance the benefit of cellular therapeutics. Her lab also conducts essential translational research, including the development of GMP-compliant cellular therapeutic products and bringing products to clinics.

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

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

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

ARTICLE & COPYRIGHT INFORMATION

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

Webinar recorded: *Dec 8 2021;* ***Revised manuscript received:*** *Feb 4 2022;* ***Publication date:*** *Feb 23 2022.*

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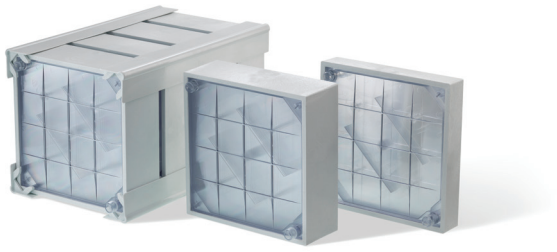

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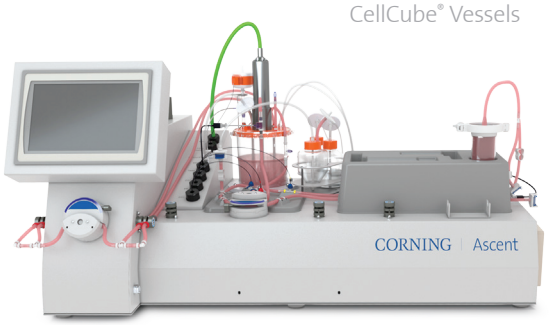

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FAST FACTS

Characterization of Roche MycoTOOL Mycoplasma Real-Time PCR Kit

Jessica Hutcheson, Scientific Project Coordinator, Rubhu Biologics

Mycoplasma contamination constitutes a serious concern for the biopharmaceutical industry. It represents one of the most common contaminants of cell cultures and biopharmaceuticals, poses a potential safety risk to patients receiving infusions of cell therapy products, and presents a significant economic risk for manufacturers due to possible batch adulteration and product recall.

Cell & Gene Therapy Insights 2022; 8(1), 1; DOI: 10.18609/cgti.2022.023

As required by regulatory authorities worldwide, all biologics manufactured in cell substrates must be tested to ensure the absence of mycoplasma contamination. Direct agar culture is the most sensitive approach and is currently an approved technique. However, nucleic acid amplification technique (NAT)-based assays are proven to be rapid, sensitive, and reproducible alternatives when validated as a comparable method of detection. They are able to detect a broad range of mycoplasma species, including non-culturable strains.

VERSATILE & ROBUST MYCOPLASMA TESTING

The MycoTOOL Mycoplasma Real-Time PCR Kit (MycoTOOL kit) is an all-inclusive reagent kit which offers fast, accurate mycoplasma testing **(Figure 1)**. It is a proven technology which can advance quality control (QC) testing strategies, and the robust design and versatility allows for fast and accurate mycoplasma detection which can support the evolving needs of cell and gene therapy developers through commercial manufacturing.

Table 1. MycoTOOL assessment studies outcomes.

Objectives	Outcomes
Optimization of the final reaction volume for qPCR using Roche MycoTOOL Mycoplasma Real-Time PCR Kit (MycoTOOL kit)	For this application, 30 µL was used as the final reaction volume (per well) for qPCR analysis with MycoTOOL kit based on results from preliminary experiments
Comparison of the sensitivity & the lowest limit of detection (LLOD) of SYBR® Green-based kit & MycoTOOL kit	MycoTOOL kit was selected for further experiments over the SYBR® Green-based kit due to the following: 1. Sensitivity as low as 1 fg (1.5 GCs of *M. arginini*) and LLOD of 5 fg (7.5 GCs of *M. arginini*) 2. Use of TaqMan probe-based assay which has higher specificity compared to non-specific DNA binding dyes such as SYBR® Green, and 3. broader coverage for mycoplasma species compared to the SYBR® Green kit.
Assessment of the recovery of spiked reference mycoplasma DNA (RMD) in non-encapsulated and encapsulated drug products	The mycoplasma detection assay in non-encapsulated drug product has a spiking sensitivity comparable to the agar culture method (10 CFU/mL) The mycoplasma detection assay in the encapsulated drug product has a spiking sensitivity comparable to the indirect cell culture method (100 CFU/mL)
Assessment of the long-term stability of spiked RMD in the non-encapsulated and encapsulated drug products	RMD spiked in non-encapsulated and encapsulated drug products at a concentration of at least 400 fg (25 CFU/mL) s stable up to 8 weeks when stored at -80°C

MYCOTOOL KIT CASE STUDY

A series of studies were performed to assess the MycoTOOL kit, with outcomes shown in **Table 1**.

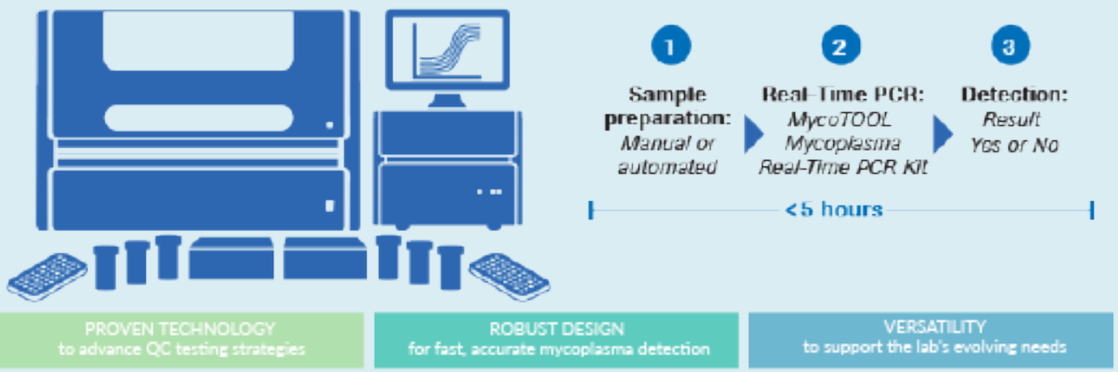
NEXT STEPS

Possible next steps for this work could involve experiments to evaluate whether higher LOD in the encapsulated drug product is due to PCR inhibition or DNA recovery inefficiency. Potentially, the unencapsulated product or encapsulation material could be affecting extraction efficiency or be causing PCR inhibition. In addition, could the 50 uL PCR reaction volume improve sensitivity for the unencapsulated and encapsulated product? The MycoTOOL kit includes a Recovery Control, which would aid in evaluating these questions.

Figure 1. Benefits of the MycoTOOL Mycoplasma Real-Time PCR Kit.

All-Inclusive Reagent Kit

- Mycoplasma DNA Free Reagents: Minimize chance of false-positive results
- Fluorescent probe-based detection: High specificity
- Universal primer set: High accuracy
- Positive control: Controls activity of enzyme and PCR components
- Recovery control: Controls DNA recovery process
- UNG: Prevent carryover contamination



CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Automating the final cell therapy bioprocess step for robust CMC/GMP compliance

Tracy Moore & Delara Motlagh

Until recently, Tracy Moore worked with the Medicines and Healthcare products Regulatory Agency (MHRA) as an Expert Good Manufacturing Practice (GMP) Inspector, specializing in sterile products and data integrity. In the following article and interview, she draws on this experience to highlight:

- Regulatory considerations and requirements for GMP compliance when manufacturing advanced therapy medicinal products (ATMPs)
- How to identify specific gaps in compliance that may occur
- Common areas of weakness found upon inspection
- The potential benefits and pitfalls of open versus closed processes

Cell & Gene Therapy Insights 2022; 8(1), 61–74

DOI: 1 10.18609/cgti.2022.033

EU REGULATORY CONSIDERATIONS & REQUIREMENTS

This article will discuss the regulatory considerations and requirements for GMP compliance, the inspection approach by the MHRA, and some considerations companies may want to take into account within their own facilities at the fill/finish stage.

Figure 1 illustrates the legislation for Europe, and it is important to note that European national content authorities are increasingly quoting Part I EU GMP as part of their deficiency references. They may also quote part IV but, this is normally for guidance only.

In the EU, the governing EU directive for medicines for human use is Directive 2001/83/EC. Within this, there is a stipulation in respect of methods of manufacture

FIGURE 1

EU regulatory considerations and requirements.

Regulation (EC) No 1394/2007 (on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004)

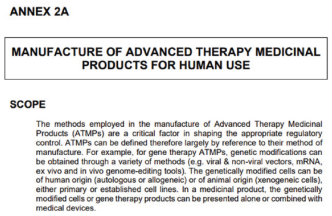
EU Tissue and Cells Directive – 2004/23/EC

EU Blood Directive – 2002/98/EC

EU GMP Part IV

National legislation for unlicensed medicines

PIC/S GMP Annex 2A



ANNEX 2A

MANUFACTURE OF ADVANCED THERAPY MEDICINAL PRODUCTS FOR HUMAN USE

SCOPE

The methods employed in the manufacture of Advanced Therapy Medicinal Products (ATMPs) are a critical factor in shaping the appropriate regulatory control. ATMPs can be defined therefore largely by reference to their method of manufacture. For example, for gene therapy ATMPs, genetic modifications can be obtained through a variety of methods (e.g. viral & non-viral vectors, mRNA, ex vivo and in vivo genome-editing tools). The genetically modified cells can be of human origin (autologous or allogeneic) or of animal origin (xenogeneic cells), either primary or established cell lines. In a medicinal product, the genetically modified cells or gene therapy products can be presented alone or combined with medical devices.

https://picscheme.org/docview/3821

EU Directive 2001/83/EC

Depending on the Member State the manufacture may or may not be captured under this Directive.

The Article relates to after a MA has been granted; however, expectations are to protect the patient.

Article 23 (1.)

After a marketing authorisation has been granted, the marketing authorisation holder shall, in respect of the methods of manufacture and control provided for in Article 8(3)(d) and (h), take account of scientific and technical progress and introduce any changes that may be required to enable the medicinal product to be manufactured and checked by means of generally accepted scientific methods.

and control that takes into account scientific and technical progress. This is under the marketing authorization (MA) section, which is article 23 (1.).

However in reality, the expectations are the same for all manufacturers of medicines, and those without an MA will come under increasing pressure to move with technical advances. This is because if we look at part IV, we can note that 'contamination' is mentioned on 77 occasions. Due to the nature of ATMPs, the aim is for it to be free from microbial contamination – section 5.10 in particular covers this well (Figure 2).

Equipment used in production or control operations should be suitable for its intended purpose, and it should not present any hazard to the product. Therefore, how a company eliminates or mitigates any risk of contamination is high on the inspector's agenda.

MHRA INSPECTION APPROACH

The following is a summary of the inspection approach taken by the MHRA. During my time at MHRA, Senior ATMP inspectors trained all the sterile inspectors in ATMPS with their years of knowledge and information built up across many inspections. The inspection approach should be no surprise however, as this is the format for all sterile products.

Quality management systems

- Change control
- Deviations/investigations
- Corrective action and preventative action procedures and action plans (CAPA)
- Validation and qualification

DOI: 1 10.18609/cgti.2022.033

FIGURE 2

EU GMP Part IV.

EU GMP Part IV

5.10. Equipment used in production or control operations should be suitable for its intended purpose and it should not present any hazard to the product. Parts of production equipment that come into contact with the product should not have unwanted reactive, additive, adsorptive or absorptive properties that may affect the quality of the product.

In addition, parts of the equipment that come into contact with cells/tissues should be sterile.

- Risk assessment

Facilities

- Design and qualification
- Contamination controls including environmental control
- Planned preventative maintenance

Equipment

- Purchase (to include design qualification)
- Qualification
- Daily checks and use
- Planned preventative maintenance and calibration

Materials (consumables; product contact, non-product contact & reagents)

- Specifications and purchasing
- Release for use – QC
- Storage

Process

- Controls including contamination controls!
- Changes
- Validation of process and aseptic simulation (media fills)
- Labelling design and control
- Storage requirements
- Tracking
- Release testing and procedures
- Non-conformances and complaints management

It is important to note that this list is not exhaustive by any measure. All inspections will cover the quality management system. In fact, it is one of the highest deficiency aspects of any ATMP inspection. An inspector will pay particular attention to change controls, and any deviations or investigations. What they are looking for in particular is a clear assessment of any change and whether appropriate actions have been taken in a timely manner, that nothing important has been missed, and that this has been reviewed by the appropriate members of the team.

Looking at facilities, and the design and qualification, the key points around this are that the facility is designed for the process under which you are manufacturing this product, and that the contamination controls – which includes the environmental controls – are appropriate and robust. In addition, with any facility, the Planned Preventative Maintenance (PPM) should be available as a schedule and staff must be complying with that schedule and performing all the right checks and balances at the right time.

Moving on to equipment, the purchase of equipment is often overlooked. How do you know what you want to buy? Does this piece of equipment fulfil your needs for manufacture of this product? If you don't have the user requirements specification (URS) and design qualification (DQ) in place, you are not necessarily going to buy the correct equipment for the process.

All equipment requires appropriate qualification. One particular point of concern for inspectors is the daily checks on equipment used, be that for an open process or a closed process. And again, that the PPM and calibration is appropriate, in place, and being adhered to.

Materials and consumables management are concerns for inspectors, both product contact and non-product contact. These need to be listed so that you know exactly the grade and/or specification that you are purchasing. Additionally, the product contact materials must be sterile, so in your purchasing specification make sure this is extremely clear. For materials and consumables that are considered critical, which includes product contact ones, ensure that these are going through your quality control system and are formally released for use, and that any storage of those materials does not adversely affect future use.

For the process, the headline is once again contamination controls. The validation of the aseptic process is always of interest, and depending on the product, different approaches are taken. However, the approach taken must be documented and justified. Any changes to the process should be put through the change control system and be approved prior to making any changes, ensuring all of the required personnel have inputted, this often includes production and engineering and not just QA staff.

Validation of the aseptic process simulation is required for both open or closed processes. Labelling design and controls is often overlooked. Labels generated are to be produced in a controlled fashion and reconciled. The storage should not be detrimental to the product, so active measures to protect the primary pack should be taken.

Tracking and traceability are clearly important for autologous products, and your systems need clear robust procedures to control this.

Concerning release testing and procedures, what is appropriate for autologous products is not necessarily the same for allogeneic products, as a greater amount of time is usually available to perform what would be considered as full pharmacopeia testing. There would be a justification required as to why the required tests could not be performed.

For non-conformances the expectation is that you would raise a deviation and investigation into a non-conforming result and establish a root cause, or a probable root cause at the very least. Complaint management processes should feed into the quality system and should not sit outside of it. They are also required to be tracked and trended.

OPEN VERSUS CLOSED PROCESSES: RISKS & BENEFITS

Of the three main areas of weakness often seen at ATMP inspections, contamination and cross contamination controls is often the largest. Quality Management System (QMS) robustness and equipment calibration and qualification are the two other common issues.

Regarding the QMS and its robustness the change control, the investigations, the root cause analysis associated with those investigations, and the CAPA plan are recurring themes. It is common for the CAPAs to be overdue, or ineffective. This is closely followed by equipment calibration and qualification which often has not been done at all, or not done correctly.

Shown in Figure 3 are 'decision trees' from the guideline on the sterilization of the medicinal product, active substance, excipient and primary container, EMA/CHMP/CVMP/QWP/850374/2015 (effective October 2019).

DOI: 1 10.18609/cgti.2022.033

FIGURE 3

Decision trees for sterilization choices for aqueous and dry power, non-aqueous or semi-solid products.

Figure 1 Decision tree for sterilisation choices for aqueous products

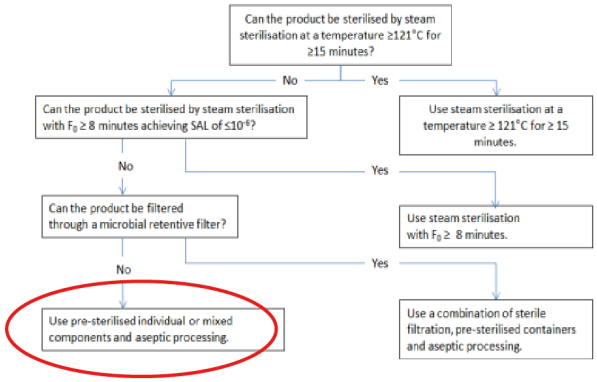
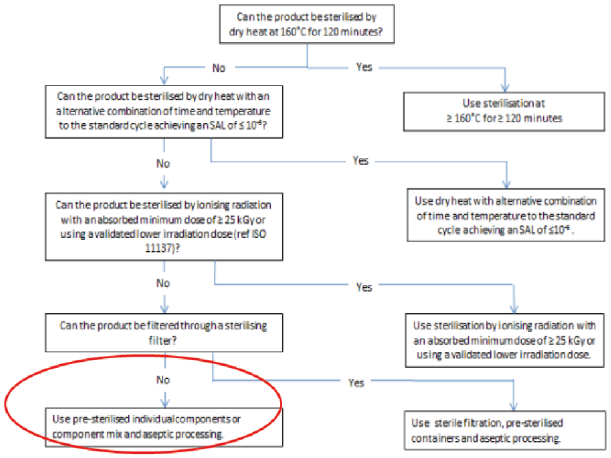


Figure 2 Decision tree for sterilisation choices for dry powder products, non-aqueous liquid or semi-solid products



Looking at the decision tree for sterilization choices for aqueous and dry powder, non-aqueous or semi solid products, the only significant mention of ATMPs is in this section states that the majority of ATMPs cannot be terminally sterilized. In such cases, the manufacturing process should be conducted aseptically. It is therefore down to the GMP inspectors to assess and consider what is acceptable in terms of the risks associated with the aseptic process.

How do inspectors assess this risk to aseptic processes? It is generally accepted that terminal sterilization is the least risky way to produce a sterile product, although it is still not without risk. Sterility of a product must be achieved, regardless of the manufacturing process, as sterility is a critical quality attribute for all sterile substances, products, and containers. This cannot be assured by testing – it needs to be assured by the use of suitably designed, validated, and controlled manufacturing processes.

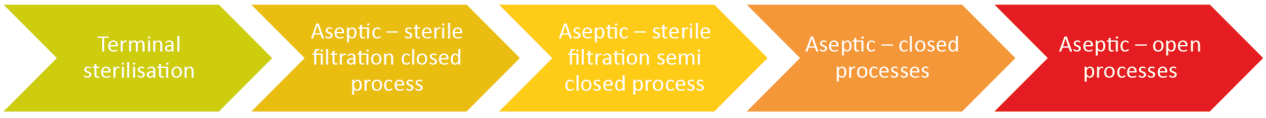
This is achieved by controlling several factors such as bioburden, the sterilization process and procedures, the integrity of the container closure system, and in the case of aseptic processing, the use of a satisfactory aseptic technique.

A note of caution here: the aseptic technique is critical in open processes, while in closed processes aseptic connections and that the system remains closed are critical. These are the areas that will be heavily focused upon by an inspector.

By virtue, it is clear that a closed system is less risky than an open one. For ATMPs, and in particular cell therapy products, this is at the highest end of risk since the medicinal product cannot be terminally sterilized or

FIGURE 4

Level of risk associated with manufacture of sterile medicinal products.



sterile filtered (Figure 4). However, risk can be reduced by having it manufactured and filled within a closed process. The obligation of the manufacturer of any medicinal product is to ensure the least risky process is used.

Manual/semi-automatic operations

When considering manual operations and semi-automatic operations i.e. 'open' processes, there are a number of key considerations for minimizing contamination events. These include the design of a process, equipment, facilities, utilities, the conditions of preparation, the addition of buffers and reagents, sampling, and training of the operators.

Breaking this down further, manual operations require a traditional Grade A/B requirement, which means full part 1 GMP Annex 1 requirements need to be in place. Because of this, the gowning and contamination controls for operators are heavily scrutinized. Are the gowns sterile garments, what is the operator aseptic technique like, how are they operating within the biological safety cabinet? Is there just one operator at the cabinet, or two? When the smoke studies have been done, have both operators been standing there, or is it only one, and have they got their arms resting on the extract grilles? There many aspects associated with the use of cabinets that need to be considered.

The location of the biological safety cabinet is also important, along with how that room can be cleaned. Can behind the biological safety cabinet be cleaned, or is it right up against or sealed to the wall? There are also many consumables associated with these kinds of processes, which are quite often in the same room. How is that room cleaned, with all these items there? There is a requirement in GMP that the area is able to be cleaned and doesn't contribute to contamination risk factors.

Placement of particle monitoring heads and environmental monitoring plates are also key. If you are getting data and it is showing zeroes, something to check straight away is where you have your environmental monitoring plates and particle monitoring head. The point of these measurement types is to capture the environment that your product is seeing.

Finally, there are process simulation tests. How are you designing that process, and are these media fills simulating those processes? The approach you are taking and the rationale behind it must be documented and fully justified.

Automated closed processes

For automated, 'closed' processes a risk assessment is required to justify the cleanroom classification based upon the equipment you are going to use and the capabilities that it has. It is generally accepted that a grade C area for closed systems is acceptable. However, the risk assessment is not simply a box-ticking exercise.

A quality-risk management approach with appropriately qualified and multidisciplinary teams must be adopted. This requirement is also applied to small companies – it is not related to company size.

The failure modes that come out of this risk assessment are to drive the daily checks, calibration and maintenance, and requalification requirements – not the other way round. You cannot specify what daily checks you want to do and make the risk assessment fix that need.

There are a number of other pitfalls to be aware of. If we consider a traditional manufacturing process, it is usual to scale up from development. With ATMP autologous processing it is normally scale out rather than scale up, because there are multiple patients with products that are patient specific, and starting materials come from them and are to be returned in product form to be administered only to them. Therefore, traceability and separation of those activities is required.

When using tubing sets things to consider are; what certification has been provided, what is the frequency of the manufacturing testing of these, and is this considered

DOI: 1 10.18609/cgti.2022.033

robust enough? Consider what frequency of tubing sets are tested within that batch. Is it five out of every hundred, or is it one out of every ten? You need to know and justify whether the frequency is robust enough for your process. Air filters are often required, depending on the machine, to maintain a closed system. These require pre-use and post-test filter integrity testing, and have been known to fail.

If you have any problems with tubing sets leaking or air filters that have failed integrity tests, the supplier should be notified via a complaint and required to investigate. There needs to be follow up to understand the impact of the issue on the process.

Depending on the equipment process design, sterile welding will almost always be required. Whilst this is considered a robust technology, bad welds do occur on occasion and checks are required to be performed. Key points for the inspector include things like single use of the razor blade or wafer. Daily checks inspectors would expect to see at the start of day include inspecting the weld on the tubing by eye on a gross check level to ensure there are no obvious defects before using it.

Leak tests involve applying air pressure and looking for bubbles (like you would with a bicycle tire). At more mature manufacturers, a pressure leak decay test may be performed. On an annual basis, there would be a requirement or expectation that that the weld machine would be sent away for more sophisticated qualification, such as the weld tensile strength. This is where pressure is applied until the weld breaks. Other qualification tests include testing the welds on all of the tubing types, on dry-to-dry tubing, dry-to-wet, and wet-to-wet tubing welds.

A high level of bioburden testing and robust method validation are also required. Quite often, sites will take spent media and use it in a crude test for bioburden, as part of the release. This does not replace a fully validated sterility test.

For cell-based products, due to the characteristics of cell therapy and their short shelf lives of around seven hours, the pharmacopeia Europa method 2.6.27 can be applied. This allows an automated growth method by BacT alert test in place of sterility. However, is it important to stress that justification is required for this approach.

DATA INTEGRITY

Any equipment, be it laboratory equipment or manufacturing equipment that has software, will require a data integrity risk assessment and require a company to put appropriate controls in place.

This includes issues such as unique user and password requirements associated with software. For example, it is not acceptable to have 'user one', 'user two' and so on, in a way that is not specific to an identified user.

Another issue is locking of methods to users. Once you have developed your method it must be locked, so users cannot amend it. The administrator who performs the locking of the methods, and may be adding or removing users, must be separate from the manufacturing team to avoid conflict of interest. Administrators are authorized to make any such amendments or deletions, but those rights cannot be applied for general day-to-day users.

Finally, audit trails must be considered. What is on that audit trail, what are you capturing, when is that checked, and how is it archived? An effective way to treat audit trails is to review them after each use, ensure that the right method has been used, that the times correlate, that there has been no amendment or deletion of that method, and that the right operator or user is on the audit trail.

CASE STUDIES: TWO EXAMPLES OF SERIOUS DEFICIENCIES

Below are examples of two serious deficiencies which halted production of ATMPs. Both are applicable to either manual and open or automated and closed processes, and both concern environmental monitoring activities.

Serious deficiency #1: lack of facility & process control

In case one, there was considered to be an ongoing lack of facility and process control. There were continued, repeated high numbers of environmental monitoring excursions when the facility was in operation and batches were being manufactured. When the company put corrective actions in place, this was unsuccessful in resolving the root causes and achieving a state of control. However, they carried on manufacturing regardless, and continued to get high numbers of EM excursions, resulting in a serious deficiency being given.

Serious deficiency #2: approach to contamination events

The second example concerns release of an advanced therapy investigational medicinal product (ATIMP). The company had several environmental monitoring excursions during manufacture. It was justified that any contamination of product would have been identified by visible growth within 24 hours of any contamination event.

This approach was wholly inappropriate. It was stated that it was based on a study that only utilized laboratory adapted American Type Culture Collection (ATCC) strains. The environment isolates that were routinely captured, and slow growing organisms that were also contained within that flora, were not included. This is despite the fact that they are likely to be found in contaminated product if they are in the environment. This company was subjected to a higher level of MHRA oversight. MHRA oversight in this regard meant the Inspection Action Group, which looks at potentially suspending manufacturing activities or even revoking a license.

CONCLUSION

When manufacturing ATMPs, there are a number of pitfalls that manufacturers must be aware of in order to meet regulatory guidelines and GMP requirements for the fill/finish stage. Being aware of common weaknesses found upon inspection, and closely considering the areas and aspects that inspectors will pay close attention to, can help manufacturers to meet the standards required to produce medicines.

ASK THE EXPERTS

Delara Motlagh, General Manager, Cell Therapy Technologies, Terumo Blood and Cell Technologies **speaks to Tracy Moore,** EPiC Auditors (Ex- MHRA Expert Inspector)

DOI: 1 10.18609/cgti.2022.033

Q **DM:** As you consider the different unit operations in manufacturing, what do you think are the biggest risks in the fill and finish step?

TM: Probably the method of fill and finish. Obviously it is a sterile product, and sterility is key. Products such as cell therapies cannot be sterile filtered, and therefore the fill and finish, and how that product comes to be in its final container, has to be in an environment that ensures there is no detrimental impact on that product.

It is all about maintaining that sterility angle. It is not about testing it at the end to make sure it's sterile, it's about putting all the arrangements in place to ensure that sterile environment is in place. That environment obviously includes whether it is in a biological safety cabinet for open processing, or in a closed system within perhaps a grade C environment. During all of those aseptic manipulations, people need to be kept away from that product, because they are the biggest contaminants.

Q **DM:** As you think of these risks, what are ways that they can be mitigated?

TM: Have a well-designed and thought-out process, and make sure that the equipment and the facilities have the required qualification performed. Make sure that environment is of the right standard, be it grade A, B, or C.

Make sure that the people are fully trained. Even with closed processes you still have operators, although they are performing a different kind of operation. Make sure that the baseline understanding is that this product needs to be sterile, and it is treated accordingly.

How you mitigate those kinds of risks is through training and education, and through making sure the process is well defined. Have really good procedures in place, describing to the operators the requirements on them. Finally, make sure that there is some kind of monitoring in there to ensure those processes are doing what was intended, and that the operators are acting in the way that they should.

This is where the quality assurance of these processes comes in – independent oversight of the arrangements to make sure that they are appropriate and meet their intended purpose.

Q **DM:** How do you see the role of automation in helping to mitigate some of these risks?

TM: Earlier I mentioned the risks around people. What automation does is remove, to a greater extent, the people element associated with aseptic processing.

That is important because the people are the biggest contamination risk in any aseptic processing. By providing automation you are removing the biggest possible contaminant.

I am not saying that is 100% assurance, because there are weak points in all processes. It's how you mitigate each of those weak points that is important. But certainly automation forms a big, fundamental part of removing people from the process.

Q **DM:** What do you see as the implications for GMP compliance as you look at these different risks and mitigations?

TM: When I think back to the deficiencies we had while I was at MHRA, a lot of them were around environmental monitoring and people understanding their environmental monitoring.

I discussed earlier the importance of making sure the particle monitoring and environmental plates are in the right position. The natural reaction is to move things out of the way while you are trying to operate in an open process. But in fact the particle monitoring, the environmental plates, need to capture that activity (whilst not causing an obstruction or hindering the operation).

It is a huge compliance risk. If you are in an open processing situation, and you are not getting good data from the environmental monitoring points, you are unaware of the risk to that product and therefore the risk to the patient. You are blissfully unaware that you have a problem. That is a huge negative from a GMP compliance perspective, and an inspector will always, always look at that.

With a closed system it is very much about the checks and balances that you do on a daily basis, and how that process has been designed. It doesn't have the same risk points, or severity of risk, but there are risks just the same. In these cases they would look at things like the aseptic connections of the tubes, for example, and how you then are going to perform a process media fill for the closed system.

From a GMP compliance perspective, between open and closed processes the actual approach is different, because the risks are different. And from an open processing perspective, the environment and the people in that environment carry the biggest risk.

Q **DM:** I really like the framing around the safety of the patient being of paramount concern; this is obviously the whole reason these therapies are being developed. Knowing what you know, and understanding both the regulations and also the environment and some of the challenges: if you could design an ideal solution for the fill and finish step, what would be the top three attributes this solution would have?

TM: They would be a facility and equipment that were qualified appropriately, a process that is closed because it is the least risk, and that the aseptic connections have minimal people contact.

You have things like the sterile welds I mentioned above. And then the closure systems, and how that product is collected at the end of the process, is again aseptic, and as closed as possible when you are removing that final product. That is how I would design it.

That takes into account, again as I was talking about earlier, the need to move with the scientific and technical advances. There is equipment out there that does this. So if you are

DOI: 1 10.18609/cgti.2022.033

going to design it, you wouldn't design the riskiest process; you would design the least risky process.

Q **DM:** Also to that point, what guidance would you provide to manufacturers and developers on when to consider automating this step in the process as they progress through the stages of development?

TM: I would say as early as possible. The risk to the patient is what is of concern to inspectors, and to companies as well. Why wouldn't you introduce the least risky process for the patient as soon as you possibly can?

Additionally, if you're starting from scratch with an open process you have to have grade A, grade B, multiple operators, the gowning, the sterile garments, the comportment training, all of the other environmental considerations associated with that, the room and facility, the upkeep of the biological safety cabinets, and the cleaning. Some of that is still required for a closed process but it is not at the same scale. So if you are going to start from scratch, start with the least risky process, and with the minimal operator intervention into that sterile process.

You should also consider data integrity. If you are going to buy equipment, make sure it meets data integrity requirements. There is data integrity guidance out there from the Pharmaceutical Inspection Co-operation Scheme (PIC/S) from WHO, and also from MHRA. If you want any pointers on what that equipment needs to do from a data perspective, there is plenty of guidance out there for people to look at too.

Q **DM:** Data management is clearly important for process analytics as well as compliance. There is a growing concern in Cell & Gene Therapy about regulations for manufacturing of these novel therapies and how they may evolve. What is your view?

TM: I can't imagine an evolving situation. The requirements have always been that if you are producing a sterile product that it is sterile. There has never been any dispensation on that.

Although they are new and emerging product types, it doesn't actually change the GMP requirements. GMPs are very straightforward – if it's a sterile product, it needs to be sterile. Now there are some changes to EU GMP Annex 1, which is sterile manufacturing, and certainly that's what MHRA and a number of EU authorities inspect ATMPs to.

The update to Annex 1 is a clarification. There are no new requirement per se, although because it's also going to PIC/S, which is the global network, there are some murmurings across the globe that they'll see them as new expectations. But certainly within Europe they are not.

If anything has changed, it is probably the experience, knowledge and understanding of the inspectors seeing these processes. Not all Competent Authorities have specific ATMP

inspectors; they have sterile inspectors and non-sterile inspectors. Depending on the processes you see, that is your knowledge and education. However, when I was in the agency it was really good at using that knowledge and sharing it across not just the individual regulator authorities but also across the PIC/S network.

For example, in November 2019 there was an event on Annex 1. Myself and Ali (Abdelaali Sarakha) from Agence nationale de sécurité du médicament et des produits de santé (ANSM), and a couple of the others like Matt Davis of the Australian Therapeutic Goods Agency (TGA), spoke to the other regulators about Annex 1 sterile processing. The specifics that you then talk about in the margins is how the inspector knowledge grows and is understood. There are more and more of these facilities, and the education and the knowledge will grow with both the regulators and the companies.

What you also have to remember is that not all companies will have been exposed to a regulator or an inspector, so they don't know what they don't know. Sometimes when you are just reading guidelines or requirements it is not entirely clear what is required. It is only through that experience of inspection that these companies can fully understand what is expected of them.

Q **DM:** You make an excellent point - this is an unknown area for many companies. For many of the manufacturers this may be their first exposure to some of these regulations as they're trying to commercialize. With that in mind, what other guidance would you give to developers and manufacturers of cell and gene therapies?

TM: You start with the end in mind, which is quite a famous phrase.

It is about good design of process. It is not about gold plating, it is about minimum, basic GMP requirements. This product needs to be sterile. With cell therapy, you can't sterile filter. Therefore, how am I going to maintain sterility without that additional step that gives me some assurance?

It is really about putting down that process and making sure that at the point of transfer you are not open to risk of contamination. That is as basic as I can make it.

BIOGRAPHIES

Tracy Moore, EPiC Auditors (Ex- MHRA Expert Inspector)

Tracy Moore is an accomplished quality professional with over 32-year experience of pharmaceutical manufacture, distribution and regulation gained from working within the industry and also for the GMP Inspectorate of the UK medicines regulator (MHRA) where she reached Expert Inspector level/status. During her 10 years as a GMP Inspector Tracy had responsibility for licensing and inspection of pharmaceutical manufacturers and distributors both in the UK and overseas, the development and implementation of regulations and European guidelines relating to pharmaceutical manufacture and distribution, the management of risk and noncompliance in these sectors and collaboration with other

DOI: 1 10.18609/cgti.2022.033

European and International regulatory authorities. Tracy has expertise across most dosage forms and in particular, sterile manufactured products produced aseptically for which she has presented on a range of topics associated with GMP Annex 1. Prior to joining the MHRA Inspectorate, Tracy spent 22 years working in both commercial and R&D environments of the pharmaceutical industry in various QA, QP and management roles covering a wide range of sterile and non-sterile product dosage forms. In addition to this she has been responsible for the audit and oversight of Contract Development and Manufacturing Organisations (CDMO), component suppliers, and API manufacturers.

Delara Motlagh, PhD, MBA, General Manager, Cell Therapy Technologies, Terumo Blood and Cell Technologies

Delara is the General Manager of Cell Therapy Technologies at Terumo Blood and Cell Therapies, headquartered in Lakewood, Colorado. She is passionate about the cell & gene therapy market and the potential these innovative therapies hold to improve the lives of patients. She brings more than 18 years of experience in biotechnology and healthcare in various therapeutic areas including oncology, cardiology, orthopedics, hematology, and nephrology. Prior to joining Terumo Blood and Cell Therapies in 2017, Delara served in diverse leadership roles at Baxter Healthcare in marketing, research & development, and operations. Her cross-functional background provides a unique perspective and deep understanding of development, cell manufacturing, and commercialization elements in the industry. Delara received a PhD in Physiology and Biophysics from the University of Illinois, fellowship in Vascular Tissue Engineering at Northwestern University, and Executive MBA from Kellogg School of Management.

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *Dr Motlagh is and employee of Terumo Blood and Cell Technologies.*

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

ARTICLE & COPYRIGHT INFORMATION

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

Webinar recorded: Jan 11 2021; **Revised manuscript received:** Dec 15 2021; **Publication date:** Feb 2 2022.

DOI: 1 10.18609/cgti.2022.033

Advances in AAV process development

"Overall, we have been very pleased with the regulatory agencies and how they are approaching gene therapy. It's a very rapidly evolving space, and they're trying to learn and work with manufacturers to make these therapies work better.
If your organization is trying to pursue an accelerated pathway [...] you need to pull in some later-stage activities earlier in the development than you normally would. Organizations need to be ready for this – if you think you're going to be trying to register on Phase I data, you need to prepare to do a lot of these BLA-enabling activities at your IND stage."

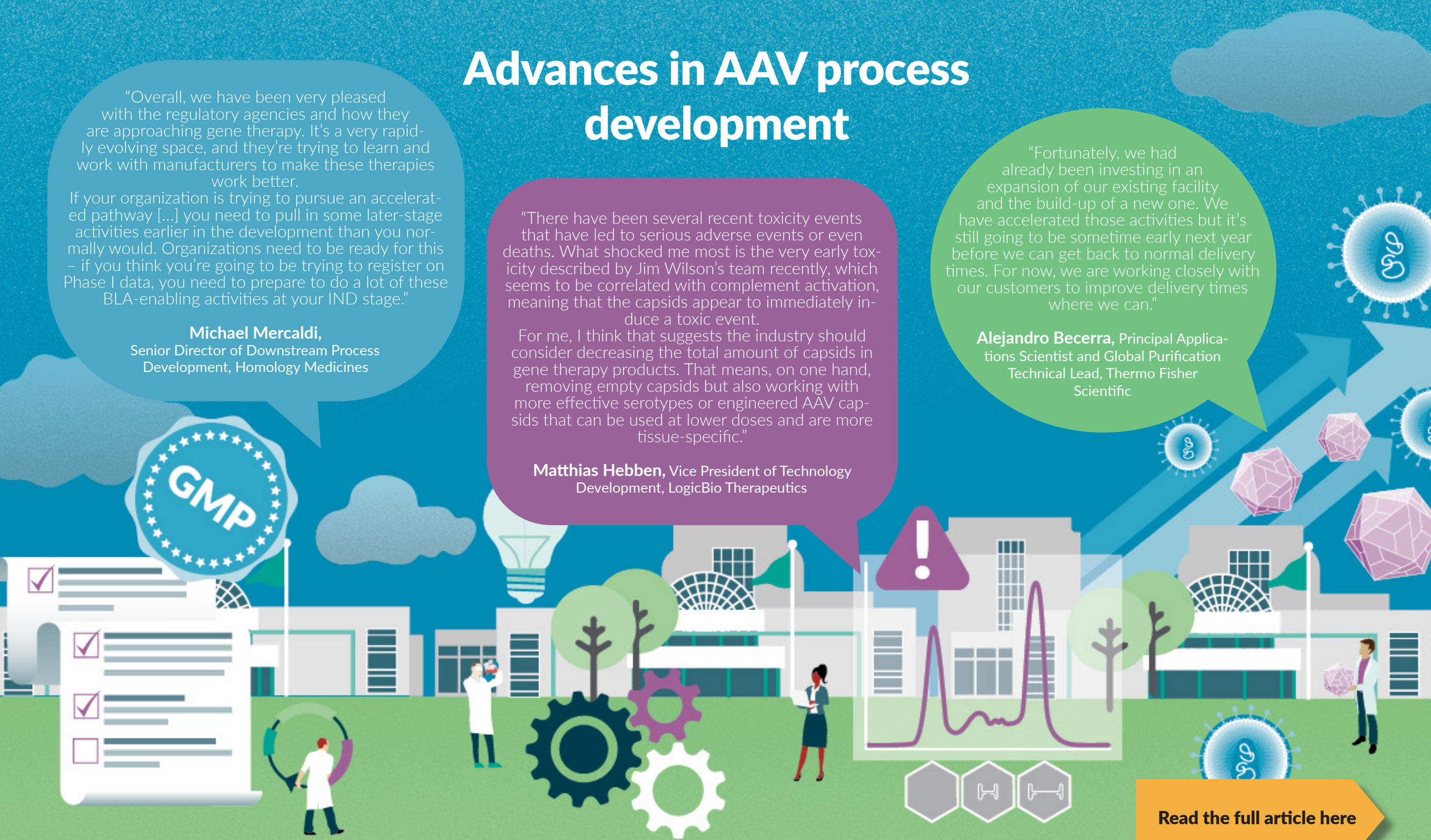
Michael Mercaldi,
Senior Director of Downstream Process Development, Homology Medicines

"There have been several recent toxicity events that have led to serious adverse events or even deaths. What shocked me most is the very early toxicity described by Jim Wilson's team recently, which seems to be correlated with complement activation, meaning that the capsids appear to immediately induce a toxic event.
For me, I think that suggests the industry should consider decreasing the total amount of capsids in gene therapy products. That means, on one hand, removing empty capsids but also working with more effective serotypes or engineered AAV capsids that can be used at lower doses and are more tissue-specific."

Matthias Hebben, Vice President of Technology Development, LogicBio Therapeutics

"Fortunately, we had already been investing in an expansion of our existing facility and the build-up of a new one. We have accelerated those activities but it's still going to be sometime early next year before we can get back to normal delivery times. For now, we are working closely with our customers to improve delivery times where we can."

Alejandro Becerra, Principal Applications Scientist and Global Purification Technical Lead, Thermo Fisher Scientific



Read the full article here

Cell & Gene Therapy Insights **and Thermo Fisher Scientific brought together a panel of experts to discuss how viral vector manufacture has evolved over the past 2 years, the challenges created by the pandemic, and what we can expect in the future.**

Cell & Gene Therapy Insights 2022; 8(1), 59; DOI: 10.18609/cgti.2022.030

CELL & GENE THERAPY INSIGHTS

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Abeona Therapeutics evaluates a scalable suspension-based platform for production of viral vector for MPS-IIIA

Anne MacIntyre, Senior Scientist, R&D BioProcessing at Pall Corporation **& Phillip B Maples,** VP Process Development at Abeona Therapeutics

Viral vector-based gene therapies are bringing life-changing treatments to patients. One investigational therapy in development is a novel hSGSH gene replacement therapy for the treatment of Mucopolysaccharidosis type IIIA (MPS IIIA), which uses an AAV capsid to deliver a functional gene. If this therapy is approved, a scalable upstream production process is needed to provide the necessary manufacturing capacity to allow it to reach patients. In a recent webinar, new insights into scaling up production of high titer and high-quality viral vectors at clinical and commercial scales were discussed. Some key experimental results are highlighted here.

ABO-102 CLINICAL PROGRAM

MPS IIIA has no currently approved treatments and leads to early neurocognitive decline. In children, most start a rapid cognitive decline by 3 years of age, and 70% do not reach the age of 18.

ABO-102 is a novel gene therapy for MPS IIIA which introduces a functional hSGSH coding sequence. This particular AAV vector can cross the blood–brain barrier and then release the functional gene in cells, allowing them to process lysosomal material effectively.

ABEONA THERAPEUTICS CASE STUDY: SCALING UP AAV293 SUSPENSION CELLS

Pall collaborated with Abeona Therapeutics to evaluate the Allegro™ STR bioreactor family as a scalable vector production platform. The investigation assessed the scalability of a PEI-mediated transfection process between the 50- and 500-L scale for production of a recombinant AAV (rAAV) vector. The Allegro STR 50 and 500 were run in parallel during production, and bioreactor performance was evaluated based on cell growth, metabolic profile, and viral vector production.

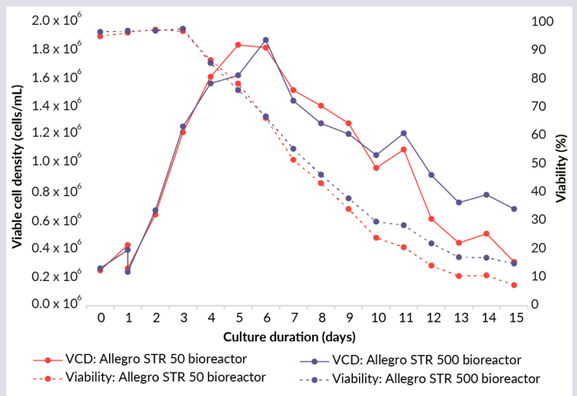
The cell expansion process took 21 days from vial thaw to inoculation of the Allegro STR 50 and 500 bioreactors, followed by a further 15 days from inoculation to harvest.

EXPERIMENTAL RESULTS

Consistent cell growth and viability trends were observed across scales, and both bioreactors achieved a peak cell density of approximately 1.8 x 10^6 cells/mL **(Figure 1)**. The STR 50 had a slightly lower viability in the second half of the culture resulting in a lower viable cell density at the time of harvest, and also consumed more glucose compared to the STR 500. The root cause of these differences was not identified, but could be attributed to slight differences in transfection efficiency between the two vessels. Lactate trends were similar across scales throughout the course of the culture.

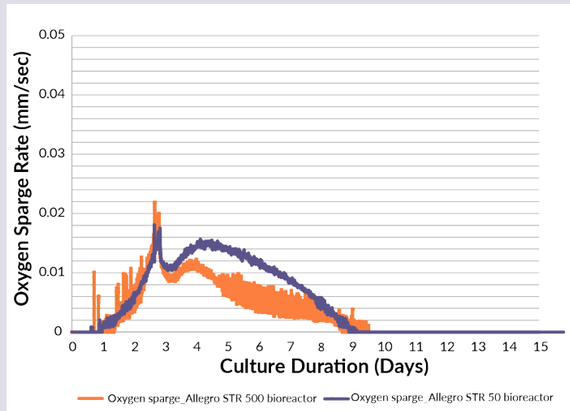
Oxygen demand was shown to effectively scale between the STR 50 and STR 500 bioreactors by maintaining a constant power per unit volume input across vessel sizes **(Figure 2)**.

Figure 1. Cell growth and viability during production in parallel Allegro STR 50 and Allegro STR 500 bioreactors.



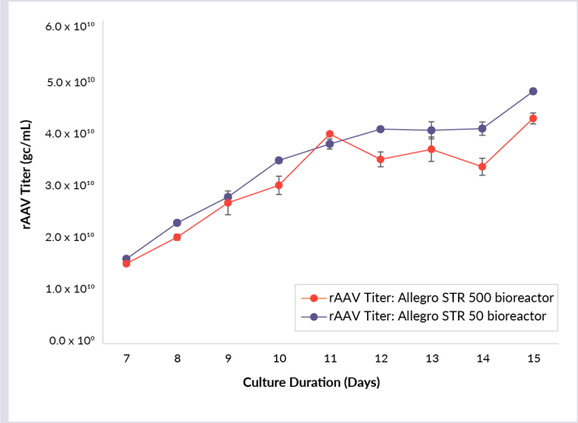
VCD and viability measured using the ViCELL XR (trypan blue exclusion)

Figure 2. Normalized O2 sparge during production in parallel Allegro STR 50 and Allegro STR 500 bioreactors.



Samples were saved for rAAV titer measurement starting on day 7 of the culture, and titer trends were similar across scales. The STR 50 and STR 500 bioreactor achieved final AAV9 titers of 4.8 x 10^{10} and 4.3 x 10^{10} genome copies (gc)/mL, respectively **(Figure 3)**.

Figure 3. rAAV titer during production in parallel Allegro STR 50 and Allegro STR 500 bioreactors.



CONCLUSIONS

Scalable upstream technologies are critical to enable the manufacturing capacity needed to bring gene therapy treatments with large target populations to market. These results demonstrate a scalable rAAV production process between the Allegro STR 50 and 500 bioreactors. rAAV titer production was similar between scales, with both bioreactors achieving over 4x10^{10} gc/mL upon harvest. Growth parameters and metabolic profiles compared well between scales.

Cell & Gene Therapy Insights 2022; 8(1), 143;
DOI: 10.18609/cgti.2022.028

CELL & GENE THERAPY INSIGHTS

Vector Channel

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VECTOR CHANNEL: ENHANCING VECTOR BIOPROCESS SCALABILITY

February 2022
Volume 8, Issue 1

FASTFACTS

FAST FACTS

EXPERT INSIGHT

INNOVATOR INSIGHT

INTERVIEW

FASTFACTS

Optimization of anion exchange purification for the large-scale production of plasmid DNA for gene therapy and DNA vaccine applications

Jenny England, Application Scientist and Innovation Leader at Thermo Fisher Scientific

Plasmid DNA has a number of therapeutic applications, and its large-scale production can be achieved in a flexible and scalable way using an anion exchange purification step. Thermo Fisher Scientific has developed a variety of anion exchange resins well-suited for plasmid purification, designed to simplify workflows and increase yield and purity. These resins use the unique POROS™ bead technology to assist in overcoming many of the challenges of large-scale plasmid purification.

Cell & Gene Therapy Insights 2022; 8(1), 51; DOI: 10.18609/cgti.2022.034

POROS RESINS FOR pDNA PURIFICATION

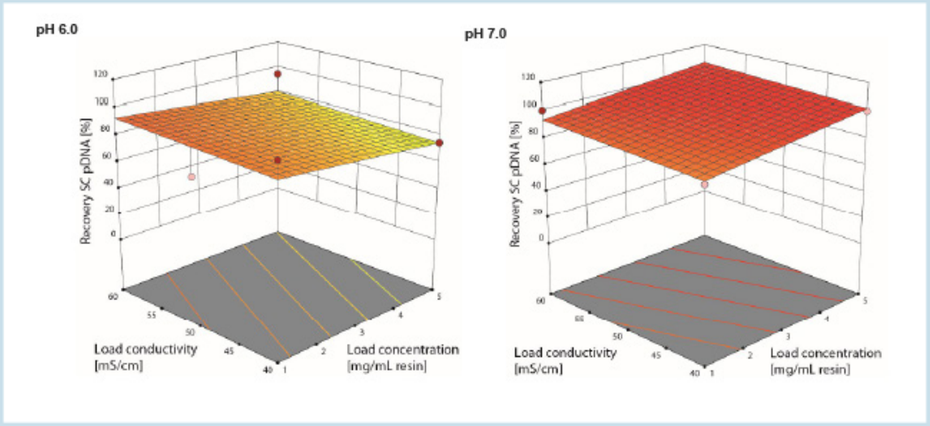
POROS™ beads were designed to overcome the low binding capacities exhibited by conventional chromatography resins for plasmid DNA (pDNA). The poly(styrene-divinylbenzene) backbone enables operation at high linear flow rates. The large pore structure reduces mass transfer resistance compared to other resins, and the small particle size improves effective impurity removal. Thermo Fisher Scientific offers a range of POROS™ anion exchange (AEX) resins, each with a unique surface chemistry and selectivity.

AEX pDNA CAPTURE DoE

Design of experiments (DoE) studies were performed on the POROS™ D50, HQ, and XQ resins with a 200 µL RoboColumn™ prepacked high throughput screening column. The POROS D50 resin (Figure 1) showed the best recovery performance at pH 7.0. At pH 6.0, loading concentration had a stronger effect on recovery from the POROS D50 resin. High recoveries were achieved at pH 7.0 and 60 mS/cm load conductivity independent of sample loading concentration.

All three AEX resins showed optimal recovery at pH 7.0 and load conductivity of 60 mS/cm (Table 1). Dynamic binding capacities (DBC) of the AEX resins were determined using a 1 mL column. Increased binding capacities were observed for POROS XQ and D50 at 2.5 min residence time and 45 mS/cm. DBC data suggests that the dimethylaminopropyl ligand along with the high accessible surface area of the D50 resin are ideal for plasmid binding. Based on the DoE and DBC results, POROS D50 was selected for scaled-up verification and further optimization.

Figure 1. POROS D50 resin recovery at pH 6.0 and 7.0.



POROS D50 SCALED-UP VERIFICATION

The binding capacity was verified on a 5.0 mL column at pH 7.0 with a 10 mg/mL loading density (Figure 2). These results confirm the binding capacity of >10 mg/mL observed in the DoE. Some pDNA was found within the cleaning in place (CIP) fraction, as shown in the gel in Figure 2. Therefore, it was identified that further recovery optimization was required to maximize binding capacity and recovery.

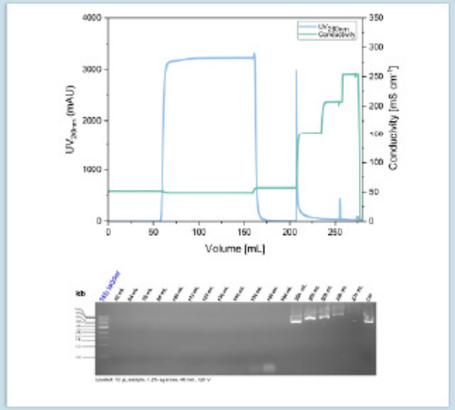
To achieve this, a small study was performed to identify optimum elution conditions for POROS D50. The recovery from the previous 5 mL scale run was not optimal and the elution conditions were changed to add 2 mM EDTA in the elution buffer, which enabled a 92% recovery to be obtained.

These results can guide further process and resin optimization for pDNA purification.

Table 1. Features of AEX POROS™ resins.

Resin	pH	Load conductivity (mS/cm)	Sample loading density (mg/mL resin)	Maximum recovery	Maximum purity	Dynamic binding capacity (mg/mL resin)
POROS D50	7	60	5	100.0	75.0	15.6
POROS HQ50	7	60	5	100.0	59.9	2.8
POROS XQ	7	60	1	100.0	73.2	9.0

Figure 2. POROS D50 scale-up.



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In partnership with: ThermoFisher SCIENTIFIC

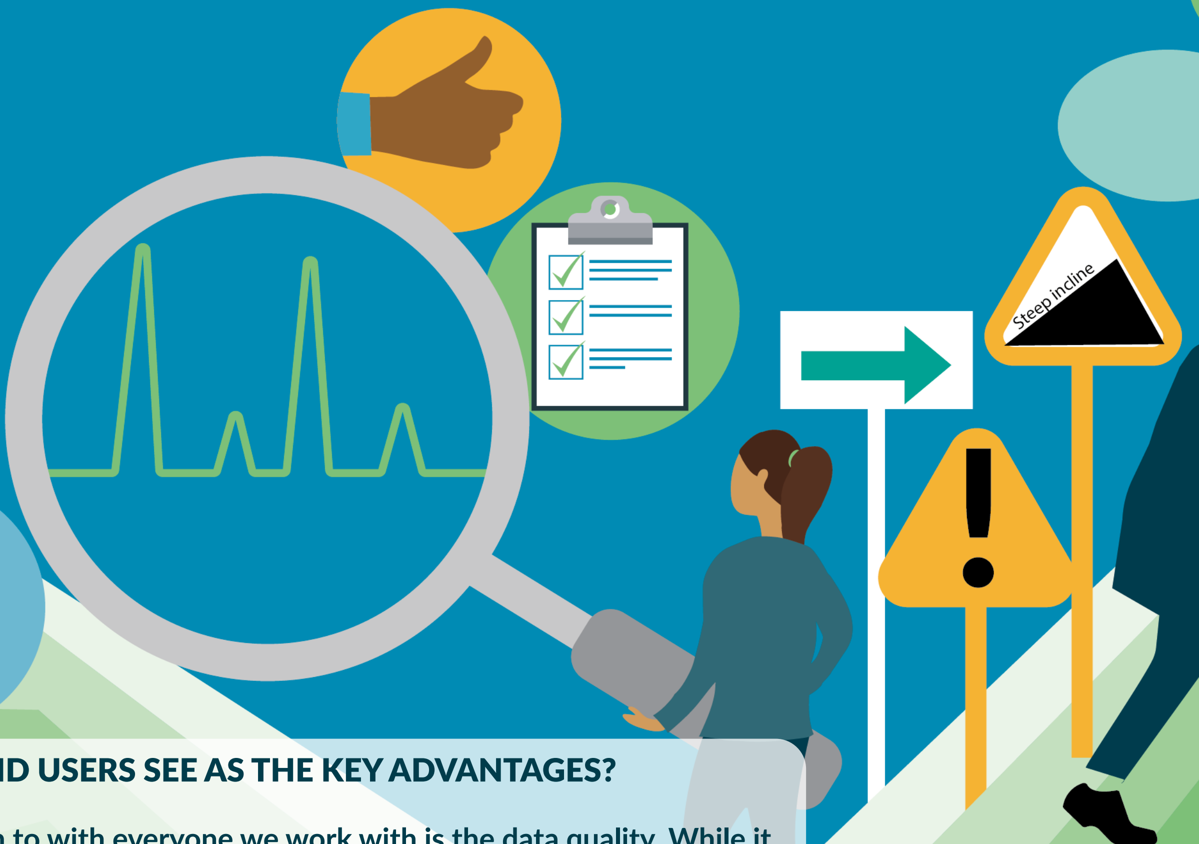
FASTFACTS

Key considerations when adopting Droplet Digital PCR for viral vector manufacture

Mark White, PhD, Associate Director of Biopharma Product Marketing, BIO-RAD

APPLICATIONS:

"Vector copy number and viral titer are the applications where people generally start their journey with ddPCR. In addition, we now offer a kit for mycoplasma detection. We're also closely watching other application areas such as residual DNA and plasmid as well as potency, with a view to hopefully supporting those in future."



WHAT DO END USERS SEE AS THE KEY ADVANTAGES?

"What it really comes down to with everyone we work with is the data quality. While it can be an additional cost to implement ddPCR over some other methods, the reliability of getting high-quality data and having tight coefficients of variation, especially when transferring from one lab to another, is key. We know many of our customers now start their process in-house and then they move it out to scale-up - having that go smoothly and quickly is so important."

"A lot of companies that have really embraced ddPCR have thought about the entire cost of ownership of the assay - not just in their own group, but for the organization as a whole as they move through the entire clinical process. That calculation really brings home why this has become such a gold standard in the gene therapy realm. In particular, where the accuracy of the viral titer measurement determines your patient dose, having that data quality is paramount."

WHAT ARE THE PAIN POINTS OF TRANSITION TO ddPCR?

"It is often the case that end users have to unlearn some things they know that are fundamentally related to qPCR, because it's a very different way of measuring. There is definitely a short learning curve, but once you are up and running, the benefits justify the transition."

HOW DOES BIO-RAD SUPPORT END USERS MAKING THE TRANSITION TO ddPCR?

"At Bio-Rad we make sure that the transition is as easy and as seamless as possible for end users. We make sure that we have really high-quality products going in, but also that for some of the newer assays that we have on market, we are developing data packages that can help guide our customers. They provide a starting point, which makes it easier for end users to replicate in their labs."

WHERE WILL ddPCR TECHNOLOGY BE APPLIED IN FUTURE?

"The obvious next step as people find the value in titer will be to look at where else ddPCR can be applied. I think we will see more and more creative ways to apply droplet digital going forward, now that it is becoming more of a widely used standard. When you do a restriction digest it all looks the same; with droplet digital, though, you are actually counting molecules. This opens up several emerging applications, such as measuring incoming plasmid quality."

HOW WILL EVOLVING REGULATORY EVOLUTION IN THE ADVANCED THERAPIES SPACE IMPACT THE USE OF ddPCR?

"There is going to be a renewed focus on the fact that it is not just about the gene of interest. The whole cassette – promoter, gene of interest, and poly(A) - is required to deliver the therapeutic molecule, and that needs to be demonstrated. I think that as multiplexing is enabled, there might be a higher level of scrutiny on those parts of the genome that are actually getting into the gene therapy, and into the patient.

The identity and integrity of your plasmids and your vectors after they are packaged is going to be an interesting area of focus in the next 5 years."

CELL & GENE THERAPY INSIGHTS

Cell & Gene Therapy Insights 2022; 8(1), 161; DOI: 10.18609/cgti.2022.007

In partnership with: BIO-RAD

CELL & GENE THERAPY INSIGHTS

ENHANCING VECTOR BIOPROCESS SCALABILITY

EXPERT INSIGHT

Commercial-scale lentiviral vector manufacturing: is the myth busted?

Hanna Leinonen

Use of lentiviral vectors in clinical trials has increased through the last decade. Lentivirus based products have entered later trial phases, and cell and gene therapy products utilizing lentiviral vectors *ex vivo* have already been accepted on market by FDA and EMA. Thus, it is no wonder that there is a demand for commercial-scale lentiviral vector production. During the last ten years, large progress has fortunately occurred in both adherent and suspension production enabling manufacturing of large quantities of lentiviral vectors in relatively non-laborious manner. In this review, different scale-up options for lentiviral vector manufacturing are described, and aspects that should be taken into account while scaling up the process are covered.

Cell & Gene Therapy Insights 2022; 8(1), 3–13

DOI: 10.18609/cgti.2022.013

With lentiviral vectors (LVs) it is possible to transduce both dividing and non-dividing cells achieving a long-term, potentially a life-long transgene expression [1]. Compared to other retroviral vectors, LVs are considered as relatively safe tools for gene therapy [2–4] and during the last 10 years, their utilization in clinical trials have more than tripled from 2.9% (in 2012) to 10.1% (in 2021) [5,6]. Three advanced therapeutic medical products for various types of blood cancers, Kymriah™, Breyanzi® and Abecma®, in which LVs are exploited *ex vivo* to modify T cells in order to express chimeric antigen receptors, have

gained marketing approval by FDA. In addition, other *ex vivo* utilized LV-based products, Skysona™ and Zynteglo™, have been accepted on European market for the treatment of cerebral adrenoleukodystrophy and beta-thalassemia, respectively. In clinical trials, LVs have successfully been used for the treatment of many other genetic diseases [7–10], and lately LV-based Covid-19 vaccines have started to be developed [11]. However, LV products applied *in vivo* are still missing from the market. Even though *in vivo* applied LV products have not been commercialized yet, they are in the pipeline.

Although for *ex vivo* applications relatively low quantities of LVs are required, the price tag of the treatment per patient can be extremely high e.g. being $475k for Kymriah and as high as $1.8 million for Zynteglo. Is it even dared to imagine how the price tag of *in vivo* utilized LV gene therapy product would look like? Of course, in addition to manufacturing costs (equipment, facility, consumables, and starting/raw materials) also labor costs, analytics, etc. are taken into account when the product price is defined. However, one of the main reasons for the high price of gene therapy products is the manufacturing, in other words, the more virus is produced per batch the lower the price could be.

For a long time, commercial scale manufacturing has been a bottleneck for viral vector based products. In commonly used adherent 2D culture systems, cells can be grown only to relatively low cell densities because in higher densities cells tend to detach. In bioreactors, higher cell densities can be achieved especially if perfusion or recirculation is applied [12,13], but in this way the total media consumption is increased, and in case of adherent bioreactors often more fetal bovine serum (FBS) is needed. Supplementing media with FBS is not only increasing the cost, but its use is question of both safety and regulatory aspects, and its availability in the future is uncertain as many gene therapy trials enter later phases, and products are accepted for commercial manufacturing [14]. There are some chemically defined animal-free adherent cell culture media and alternatives for FBS, such as Pro293™a (Lonza), OptiPEAK (Invitria) and PeproGrow-1 (PeproTech) available off-the-shelf. However, cell adaptation to new media composition may not be easy and can require further media customization.

Biggest challenges in manufacturing have been related to vectors that require plasmid transfection, such as adeno-associated viral vector (AAV) and LV. Use of traditional CaPO4 co-precipitation is relatively cheap but also very sensitive method [15]. It requires the presence of FBS to reduce cytotoxic effects, and is difficult to scale up [13,16,17]. For large virus preps, tremendous quantities of expensive high quality plasmids are needed [18]. The cost of such plasmids for large-scale production can easily be above $100k per batch. On the other hand, plasmid DNA in harvested product is a challenge for downstream processing because in final product it is considered as a safety issue [19]. Fortunately, there are good endonuclease options for residual DNA removal on the market, such as Benzonase® (Merck), Denarase® (c-Lecta), and M-SAN and SAN-HQ (ArcticZymes). However, without process optimization their price per batch can also be very high.

In 2D cultures, harvest is often performed only by collecting the supernatant and downstream processing usually covers clarification and concentration of the virus by ultracentrifugation [20]. This may be enough for small pre-clinical experiments but not for clinical trials and commercial manufacturing.

LV PRODUCTION IN ADHERENT CELLS

In many approaches for scaling up the LV manufacturing process, multiple 2D cultivation systems, such as Cell Factories™ [21], HYPERFlasks® [22], and HYPERStacks® [23], have been used. However, these only allow a modest increase in productivity, are laborious, culturing conditions cannot be fully controlled, and large incubator space is required. Corning's CellCube® system offers somewhat

DOI: 10.18609/cgti.2022.013

larger cultivation area (8.5 m^2 in one 100-layer module; Table 1), possibility to connect multiple modules to each other and to apply perfusion, and thus better support the nutrient and oxygen supply. However, they also require to be placed in an incubator [24]. In order to better control culturing conditions, bioreactors are required.

The World's first gene therapy product, Gendicine™ [25], was produced in cells attached to Fibra-Cel® disks, (Table 1) in other words, microcarriers that were packed into a suspension bioreactor. Also, LV has been produced in such system [26]. Although culturing area can be massively increased by using microcarriers (if utilized with the largest suspension bioreactors), there have been problems with clumping and cell detachment, and separation of the virus from the microcarriers (and cells) before downstream processing may be difficult [27].

Hollow fiber and fixed-bed bioreactors (Table 1) offer a solution for easier separation of the virus from the cells. In Quantum® bioreactor (Terumo BCT), cells are attached to hollow fibers instead of freely floating microcarriers [28]. Unfortunately, cultivation area in this particular bioreactor is not large being only 2.1 m^2. Fixed-bed bioreactors, instead, are more scalable, and there already are couple of options available on the market.

Pall's single-use iCELLis® fixed-bed bioreactors that contain hundreds of 13.9 cm^2 non-woven polyethylene terephthalate (PET) fibers ('carriers') provide 3D cell cultivation area ranging from 0.53–4 m^2 suitable for optimization and small scale production, up to 500 m^2 commercial scale bioreactor (66–500 m^2) in which the harvest volume can even be hundreds of liters if perfusion (or recirculation) is applied [18]. iCELLis bioreactors are available both

TABLE 1

Examples of adherent and suspension culturing system options for different production scales.

Manufacturer	Small-scale	Mid-scale	Large-scale
Eppendorf	Fibra-Cel® disks, 0.12 m^2/g (A) [1] DasBOX®, 60-250 mL (S) BioBLU®/BioFlo®, 0.1–3.75 L (S)	Fibra-Cel® disks, 0.12 m^2/g (A) [1] BioBLU®/BioFlo®, 3.3–40 L (S)	Fibra-Cel® disks, 0.12 m^2/g (A) [1]
Terumo BCT	Quantum, 2.1 m^2 (A)		
Pall	iCELLis® Nano, 0.53–4 m^2 (A/S) [2] Allegro™ XRS, 2 L (S)	Allegro™ XRS, up to 25 L (S)	iCELLis® 500, 66–500 m^2 (A/S) [2] Allegro™ STR, 50–2000 L (S)
Univercells	scale-X™ Hydro, 2.4 m^2 (A/S) [2]	scale-X™ Carbo, 10-30 m^2 (A/S) [2]	scale-X™ Nitro, 200–600 m^2 (A/S) [2]
Corning	Cell cube® 0.85–8.5 m^2 (A) Ascent™, 1–5 m^2 (A)	Ascent™, 20–100 m^2 (A) [3]	Ascent™, up to 1000 m^2 (A) [3]
Sartorius	Ambr® 15/250, up to 48× 10–15 mL (S)/up to 24× 100–250 mL (S) Biostat® RM, 100 mL–5 L (S) Biostat® B-DCU (with Univessel®) (S) [4] Univessel®, 2–5 L (S)	Biostat® RM, 5 –25 L (S) Univessel®, up to 10 L (S)	Biostat® RM, 25–100 L (S) Biostat® STR, 50–2000 L (S)
Cytiva	WAVE Cellbag, 50 mL–5 L (S)	WAVE Cellbag, 5–25 L (S) Xcellerex XDR, 4.5–10 L (S)	WAVE Cellbag, 25–100 L (S) Xcellerex XDR, 50–2000 L but wv 22–2000 L (S)
Merck	Mobius® 3 L, 1–2.4 L wv (S)	Mobius® 50 L, 10–50 L (5:1 turndown ratio i.e., 10–50 L) (S)	Mobius® 200–2000 L (5:1 turndown ratio i.e. 40–2000 L) (S)
Thermo Fisher Scientific	Hyperforma glass bioreactor, 1-3 L (S)	Hyperforma glass bioreactor, 7–15 L (S) Hyperforma Rocker, 5–25 L (S)	Hyperforma S.U.B. 5:1, 50–2000 L but 5:1 turndown ratio (i.e. wv 10–2000 L) S) Hyperforma S.U.B. 10:1 and 20:1, 50-5000 L but 10:1 and 20:1 turndown ratios depending on the scale (wv 5–5000 L) (S)

A: Adherent system; S: Suspension system; wv: Working volume.
[1]Used together with a suspension bioreactor.
[2]Mostly for adherent cells but also applicable with suspension cells.
[3]All sizes not yet available.
[4]Can be used to control up to six bioreactors independently from each other.

in low (96 g/L) and high compaction (144 g/L), and they have been utilized in production of many different viral vectors, vaccines and recombinant proteins by us and others [13,18,29–34]. To proof the scalability, we have optimized LV production such as inoculation cell density, perfusion, transfection, and harvest in iCELLis Nano bioreactors [13], and successfully performed the scale-up run using commercial scale iCELLis 500 bioreactor with 333 m^2 cultivation area, in other words, the largest low compaction fixed-bed currently available [18].

For *ex vivo* gene therapy, iCELLis Nano scale may not be enough but the large-scale production in iCELLis 500 may be too much, at least for clinical trials [18]. Unfortunately, mid-scale bioreactors, which could be more suitable for *ex vivo* therapies, are missing from the iCELLis series at the moment. Some years back, Univercells brought a competitor fixed-bed bioreactor series, scale-X™, on market, in which the mid-scale (10 m and 30 m^2, scale-X Carbo) is available together with the small-scale 2.4 m^2 (scale-X Hydro) and large-scale 200–600 m^2 (scale-X Nitro). The designer behind the scale-X bioreactor is the same who originally developed the iCELLis bioreactors [35]. Whereas in iCELLis bioreactors fixed-bed consists of hundreds of PET macrocarriers packed inside the bioreactor, in scale-X bioreactors fixed-bed resembles a double-layered roll-like structure with a spacer net between the PET membrane layers. Only one compaction is available in scale-X bioreactor series. In our study, we found LV and adeno viral vector productivity equal in both iCELLis Nano and scale-X Hydro bioreactors, with cells better distributed in scale-X Hydro compared to iCELLis Nano [36]. In addition, scale-X bioreactors have been utilized at least for vaccine production [37].

The latest invention among the fixed-bed bioreactors is Corning's Ascent™ bioreactor [38], which contains woven PET membrane, in which the cells should be more equally distributed than in other fixed-bed bioreactors. In Ascent's bioreactor, cells are cultivated in 2D manner and can be enzymatically detached. Moreover, the bioreactor uses different, less shear strategy to provide oxygen and nutrients for the cells. Similar to scale-X, also in the Ascent bioreactor series, there are or are planned to be bioreactors ranging from R&D level (1–5 m^2) through the mid-scale (20–100 m^2) up to commercial scale reaching as large as 1000 m^2.

LV PRODUCTION IN SUSPENSION CELLS

Although with adherent bioreactors, it is possible to scale up the production up to 500–1000 m^2 cultivation area, and with optimized protocol large quantity of LV can be produced [18], for certain diseases that require to be treated *in vivo*, adherent scale may not be enough. Viral vector manufacturing in suspension bioreactors very likely is the most cost-effective in a long run, as the scalability theoretically is unlimited but may require large-scale transfection optimization [39]. LV has successfully been produced in suspension cultures [12,40] Already now, there are suspension bioreactors that enable culturing ranging from very small scale bioreactors well applicable as scale-down models up to very large stirred tanks with culturing volumes of over a thousand litres (Table 1). Suspension bioreactors are available both as wave-mixed and stirred tank –type bioreactors. In addition, there are both multi-use and single-use options available. For process optimization, the options in suspension can be considered to be better than in adherent process as with e.g., Ambr® 15 and Ambr 250 bioreactors (Sartorius), it is possible to perform up to 24 (Ambr 250) or 48 (Ambr 15) parallel bioreactor runs using culturing volumes between 10–15 mL (Ambr 15) and 100–250 mL (Ambr 250) [41]. In adherent bioreactors, similar very small-scale multi-module systems are still missing.

In suspension culture, FBS is not required, and many suspension culture media

DOI: 10.18609/cgti.2022.013

on the market, such as Freestyle™ (Gibco), HyClone™ SFMTransfx-293 (Cytiva), BalanCD (Irvine Scientific), and Ex-CELL® CD HEK293 viral vector medium (Merck), are chemically defined and do not contain animal-derived compounds diminishing the quantity of unwanted contaminants in the product. However, if the production system has originally been adherent, the transfer to suspension process can be challenging. Not all the cells easily grow well and produce virus in suspension, optimization can be laborious, and nevertheless, the transfer may turn out to be unsuccessful. One reason for difficulties in transferring the process to suspension may be the FBS that is not used in suspension production, but which is beneficial at least for retroviral vector production [42].

HOW TO GET ENOUGH CELLS FOR INOCULATION?

The current bioreactor options already enable scaling up the culturing volumes up to hundreds or even thousands of litres. However, also other aspects require to be considered when process is scaled up. For small-scale bioreactors, it is easy to expand cells for inoculation for example using T-flasks or shaker flasks. In large-scale production, however, massive quantity of cells is required. In suspension process, cells can be expanded using smaller scale suspension bioreactor compared to in which the actual process is performed. For adherent process, however, cell expansion for inoculation may be more tricky. One option is to use 2D culture systems such as Hyperflasks, but for very large quantities, these are laborious. If producer cells grow both in suspension and as adherent, mid-scale suspension bioreactors can be used for expanding the cells prior inoculation of large adherent bioreactor [30]. There are also adherent bioreactors available designed to be used as N-1 bioreactors, for example, for expanding the cells for inoculation of a larger bioreactor, such as Pall's Xpansion®. In addition, as cells can be detached from the Corning's Ascent bioreactor, it is possible to use them not only for virus production but also for expanding cells for larger bioreactors.

LARGE-SCALE TRANSFECTION: THE MOST CRITICAL STEP IN LV MANUFACTURING

Success of the transient transfection [43] directly affects the yield. Transfection is one of the most expensive steps in the process, because in commercial scale production large quantities of high-quality plasmids and transfection reagents are needed. Because CaPO4 co-precipitation method is not the most suitable for large-scale transfection [13,16], other transfection strategies are required in the scale up. Electroporation is a good option for small- and mid-scale suspension cultures but is likely not the best alternative for very large-scale transfection [44]. Flow Electroporation™ technique (MaxCyte®) also works for large cell quantities, but is not applicable with adherent cultures. Polyethylene imide (PEI) such as PEIpro® (PolyPlus transfection), is a relatively cheap, less toxic reagent compared to CaPO4, and it works well not only in adherent [13] but also in suspension cultures [40]. In addition, PEIpro is available in research grade but also in high quality- and GMP-grade. We have shown that it is possible to reduce the plasmid quantity (and thus reduce cost), and optimize PEI-mediated transfection protocol in small-scale adherent bioreactors [13] and scale-up the process to commercial scale without a decrease in productivity (per cm^2) [18]. In addition to PEIpro, there are also other transfection reagents such as Lipofectamine™ (Thermo Fisher) [45], and TransIT-VirusGEN® (Mirusbio), the latter of which has been shown to yield larger titres in comparison to PEI especially in suspension [46], and which is also available in GMP-grade.

However, both of these reagents are more expensive compared to PEI.

An important aspect that should be taken into account when optimizing transfection is to think how the transfection protocol would work in a large scale. How to perform mixing, incubation and addition of the transfection mixture into the bioreactor in a manner that the method would be as similar as possible in order to have the same sized transfection complexes also after scale-up [47]? Large quantities of plasmids are required at commercial scale and therefore, an important factor that needs to be taken into account is the DNA concentration of the transfection mixture during incubation, because transfection reagents can have maximum limits for this. In addition, with adherent bioreactors that have the working volume often below 70 L, the transfection mixture usually stays in reasonable volumes. However, for >1000 L suspension bioreactors transfection mixture preparation can be more challenging.

Because transient transfection is expensive, and residual plasmids pose a risk of immune reactions in the final product [19], in optimal case LV would be produced using stable producer cell lines. Elimination of the transfection step could reduce batch-to-batch variation, because variability caused by transfection is excluded. Although a lot of development work has been done, there are still problems related to stable packaging cell lines [48]. Constitutive packaging cell lines [49] are usually yielding lower titers due to prolonged expression of toxic lentiviral genes such as VSVg, gag and pol [48]. However, good experience has been gained with LV pseudotyped with non-toxic envelope proteins [49]. For VSVg-pseudotyped LV, an option is to use inducible systems, but these have been either leaky or productivity has been low. Tet-off -typed systems in large-scale are laborious and time-consuming as often multiple complete media changes/washing steps are required for induction [50]. Although Tet-on -typed systems [51, 52] are less leaky and virus production is easier to induce as the inducing agent is added into the culture and not removed completely, more purification steps are often required in the downstream processing because the inducing agent needs to be removed [48].

CULTIVATION STRATEGY & HARVEST

While selecting the cultivation strategy, whether to use a batch or more continuous strategy, it is important to think how the short half-life LV product is harvested, and how the strategy would effect on the yield and the harvest volume, and therefore also to downstream processing. Batch mode [26] may not be the most optimal. Cells often require fresh media in order to stay in better condition, to reach optimal cell density for transfection and to produce virus longer. Advantages of batch strategy are that it is easy and quite cheap method, and the harvest volume stays relatively low. However, the yield could be larger if other strategies are used.

As a one step further from batch mode, is to harvest at multiple steps by draining the media and refilling the bioreactor. Although in this way harvest volume and likely also yield would be larger, the strategy itself is quite time consuming and laborious. It could work in adherent process, but in suspension bioreactor such strategy, especially in large-scale, is very complicated [52,53].

In order to increase cell density, feed the cells, and increase viral yields, often perfusion or recirculation is applied [12,13]. In adherent fixed-bed bioreactors, as cells stay attached to the cultivation surface, perfusion/recirculation is simple [13]. By optimizing perfusion - for example, by targeting low glucose concentrations as we have done [13] - it is possible to decrease media consumption and thus also reduce costs and minimize harvest volume without affecting the total yield.

Perfusion can also be applied in suspension cultures but as cells are not attached to any surface but freely floating in the media, separation of cells and media from each other is required. For some mid-scale wave-type

DOI: 10.18609/cgti.2022.013

suspension bioreactors membrane-based perfusion options are available (such as for Sartorius Biostat RM system). However, for larger scale, other perfusion systems are required. Alternative tangential flow (ATF), such as XCell ATF® from Repligen is commonly used with suspension bioreactors for recombinant protein production [54]. It is available both as multi- and single-use, and in different sizes that range from ATF1 applicable with 0.5–2 L systems up to ATF10 that can be used together with up to 1000 L bioreactor and when combining two ATF10 systems with up to 2000 L suspension bioreactors. However, ATF may be too harsh for fragile LV, and thus the protocol used should be carefully designed. Tangential Flow Depth Filtration (TFDF®, also available from Repligen; scalable between 1–2000 L) has been utilized for separating the cells in LV suspension process [55], and could likely also be applied in perfusion. In addition, acoustic wave systems have been used in suspension production of LV [12] and other vectors [56], but not yet in a very large scale. Also fixed-bed bioreactors have been used for suspension process [34,57]. Suspension cells are not bound to the fixed-bed but they are entrapped into it, which allows application of the same sensitive perfusion system that is used in adherent cultures in fixed-bed bioreactors. It also makes the clarification step easier, because there is no need to separate large quantity of cells from the media prior further downstream processing, as there is in traditional suspension process.

In commercial-scale production, harvest volumes can easily increase to hundreds or even to thousands of liters. Taken into account that after harvest, the product requires to be downstream processed, it is not clever to collect very dilute virus, for example, to start the collection too early and continue for too long when the titers have already decreased too much, as this can increase the harvest volume and thus the cost and labor. In our adherent process we have collected the perfused media between 24 h post-transfection and 72 h post-transfection [13], and after harvest is started media used in perfusion has not contained FBS as in that way the amount of residual FBS is reduced making the process cheaper, and importantly, facilitating downstream processing. Due to the short half-life of LV, however, a continuous process would in an ideal case be applied in which virus is harvested and immediately downstream processed without performing these two as separate steps [58].

TRANSLATIONAL INSIGHT

Although there are no *in vivo* applied LV-based gene therapy products commercially available yet, there are already options for large-scale virus manufacturing. These include adherent-based bioreactors, such as fixed-bed bioreactors, that enable LV production in small-, mid- and large-scale providing cultivation area up to 500–1000 m^2, if not quite yet then in the future. Harvest volume in such systems can be hundreds of litres depending on the protocol. Suspension bioreactors are considered more scalable compared to adherent systems, and thus will likely eventually become more common. With currently available suspension cultivation systems, production is possible to increase at least up to 5000 L. However, to become more cost-effective, suspension transfection may need to be improved. In adherent fixed-bed bioreactors, perfusion is easy to implement, as the cells are tightly attached to the cultivation surface. Even though membrane-based relatively gentle perfusion systems are available for some suspension bioreactors, with larger-scale stirred tanks, most perfusion options can be too harsh for LV process. Large-scale transfection has been shown to succeed well, and there are also relatively good stable producer cell lines available at least for pseudotypes other than VSVg.

Although it is already possible to produce LV in large-scale, the current protocols are not the most cost-effective, which directly affects the price tag of the product. To reduce costs and the price of the treatment

per patient, in an ideal future case, stable continuous suspension adapted LV producer cell lines would be cultivated in suspension bioreactors equipped with gentle automated perfusion systems that separate cells and the media that contains the fragile LV from each other. Perfused media is continuously harvested and downstream processed in a manner that the short half-life LV is not lost. However, the road to there may still be complicated. Despite the upstream part could be developed to yield large quantities of LV, the virus also requires to be purified and concentrated. Currently, recoveries especially after chromatography step are still quite low [59,60]. Thus, in order to yield large enough quantities of LV for commercial markets, it is not enough to only be able to scale-up the production – it is equally important to successfully scale-up the downstream process to obtain good recovery of the virus. Unfortunately, we are not there yet.

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DOI: 10.18609/cgti.2022.013

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

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

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

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *Nov 22 2022;* ***Revised manuscript received:*** *Jan 11 2022;* ***Publication date:*** *Jan 21 2022*

CELL & GENE THERAPY INSIGHTS

ENHANCING VECTOR BIOPROCESS SCALABILITY

INNOVATOR INSIGHT

Platform optimization for efficient AAV purification: insights from a CDMO

Vincent Ravault & Nicolas Laroudie

Over the last decade, the number of clinical trials involving recombinant adeno-associated viral (AAV) vectors has dramatically increased, the diversity of serotypes has expanded, and the demand for larger quantities of highly purified material manufactured to cGMP standards has rocketed. For contract development and manufacturing organizations (CDMOs) like Yposkesi, the manufacturing challenges are centered around flexibility, robustness, and productivity, especially with regards to purification. Universal tools able to address any serotype with minimal process adjustments are critical. In this article, we describe how POROS™ CaptureSelect™ AAVX resin can be used as a pan-affinity tool for the universal capture of AAV vectors, and how Yposkesi optimized the operational parameters to make the resin an efficient, robust, and productive purification platform that fits within the constraints faced by CDMOs.

Cell & Gene Therapy Insights 2022; 8(1), 145–158

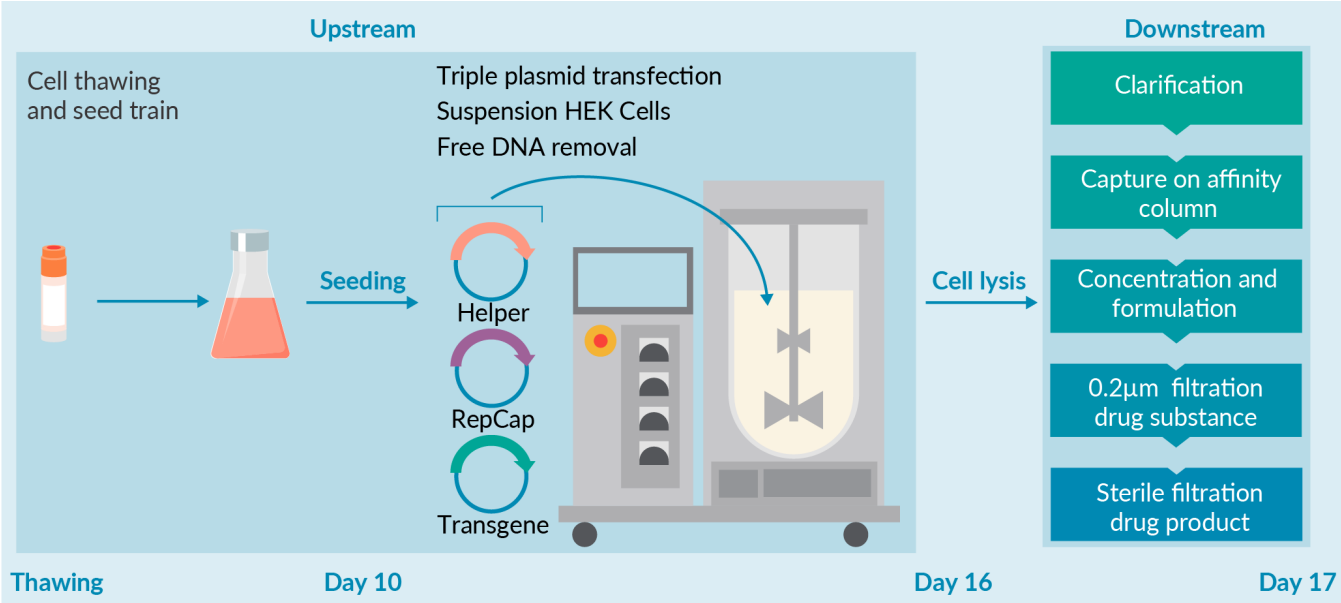
DOI: 10.18609/cgti.2022.001

As a full-service CDMO for innovative gene therapy products, Yposkesi supports customers from early-stage development, including process and analytical development, through to large-scale production and commercial supply of gene therapy products.

Yposkesi produces recombinant adeno-associated virus (rAAV) and recombinant lentivirus (rLV) vectors using adherent- and suspension-adapted cell platforms. The manufacturing platform at Yposkesi currently includes four independent production suites equipped with 200 L single-use bioreactors, which will evolve to include a 1000 L single-use bioreactor from 2023. Yposkesi is currently building an additional 5,000 m^2 clinical/commercial

FIGURE 1

AAV manufacturing process at Yposkesi.



manufacturing plant to support the growing demand for viral vector supply.

This article describes how Yposkesi developed an AAV purification platform for a range of serotypes based on Thermo Fisher Scientific's POROS CaptureSelect AAVX Affinity Resin.

YPOSKESI'S AAV MANUFACTURING PROCESS

The established AAV manufacturing process at Yposkesi is shown in Figure 1. AAV vectors are produced by triple plasmid transfection in human embryonic kidney (HEK) cells. The lysate is clarified and then directly loaded onto an affinity column. The eluted vectors are concentrated and formulated, before being sterile filtered. The full process lasts 17 days, from cell thawing to drug product filling.

The current AAV purification process involves the use of different affinity sorbents according to the AAV serotype to be produced. The POROS CaptureSelect AAVX Affinity Resin leans on the use of a ligand derived from a heavy-chain antibody that can bind AAV serotypes 1–9 and synthetic or recombinant AAV vectors, offering a great opportunity to develop the next AAV purification platform at Yposkesi (Figure 2) [1].

EVALUATION OF DYNAMIC BINDING CAPACITY

As a first step to evaluate the AAVX resin as a platform purification solution, the dynamic binding capacity was evaluated using an AAV8 serotype. The binding capacity was assessed using 1 mL-prepacked columns, packed with either POROS CaptureSelect AAV8 or POROS CaptureSelect AAVX. The binding capacity was assessed at 1 and 3 mins residence time on two different feedstocks, each with different initial virus titers.

Clarified supernatant containing AAV8 vectors was directly loaded on the affinity columns until a 10% breakthrough in AAV8 was observed in the flowthrough.

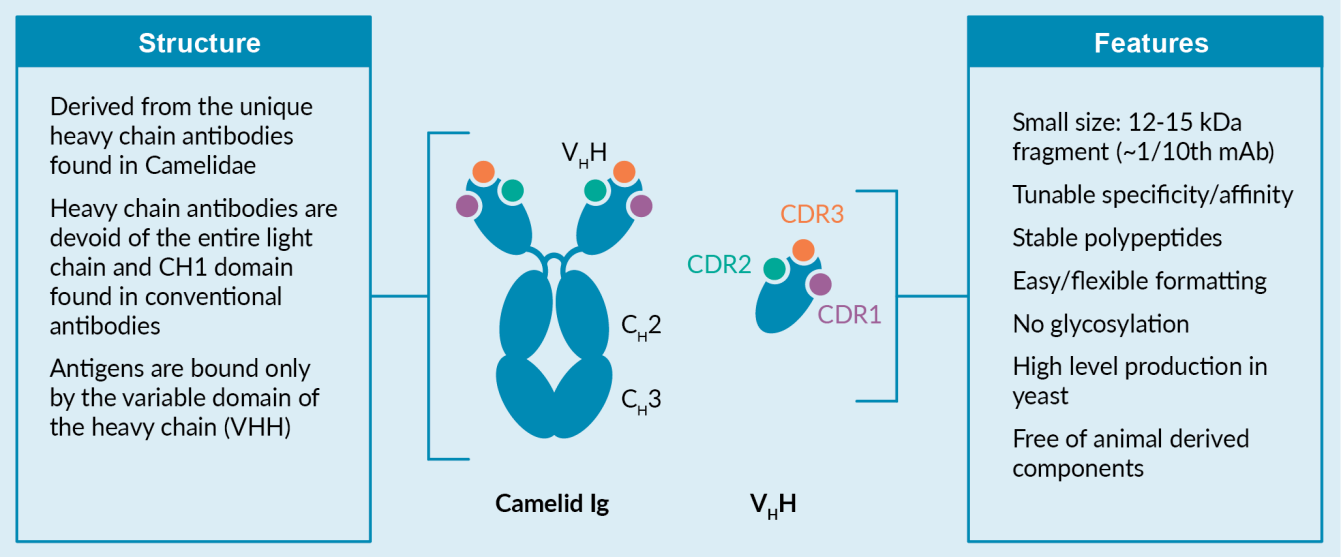
Multiple fractions (column volumes [CV]) were collected at the outlet of the column during the loading phase, and the quantity of capsids was determined by ELISA assay in each collected fraction. The results for the 3 mins residence time are presented in Figure 3.

No breakthrough was observed on the AAVX resin at loading volume of up to 1,500

DOI: 10.18609/cgti.2022.001

FIGURE 2

Key features of camelid-derived, recombinant expressed ligands used in CaptureSelect™ Affinity Resins.

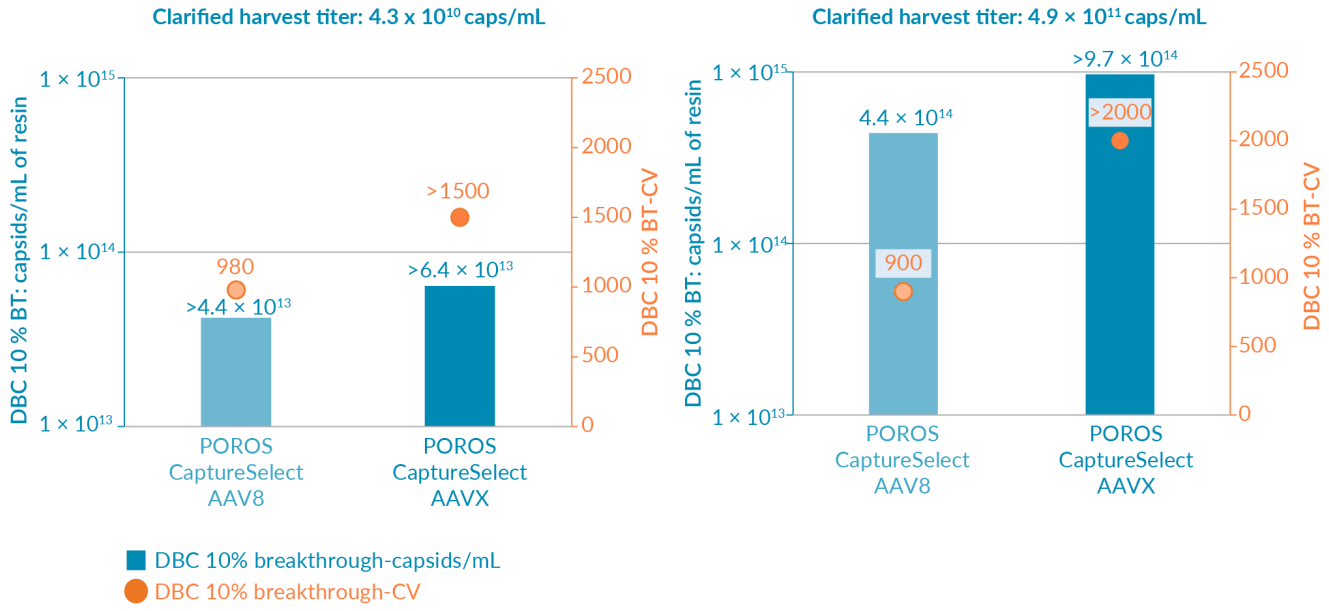


or 2,000 column volumes for the low viral titer and higher titer feedstock, respectively. Both resins showed higher binding capacity when feeds contained a higher vector titer, but overall, the AAVX resin showed a higher binding capacity for AAV8 than the Poros CaptureSelect AAV8 resin.

Figure 4 shows the binding capacity of AAV8 vectors measured at 1 min residence time on both resins, showing similar binding

FIGURE 3

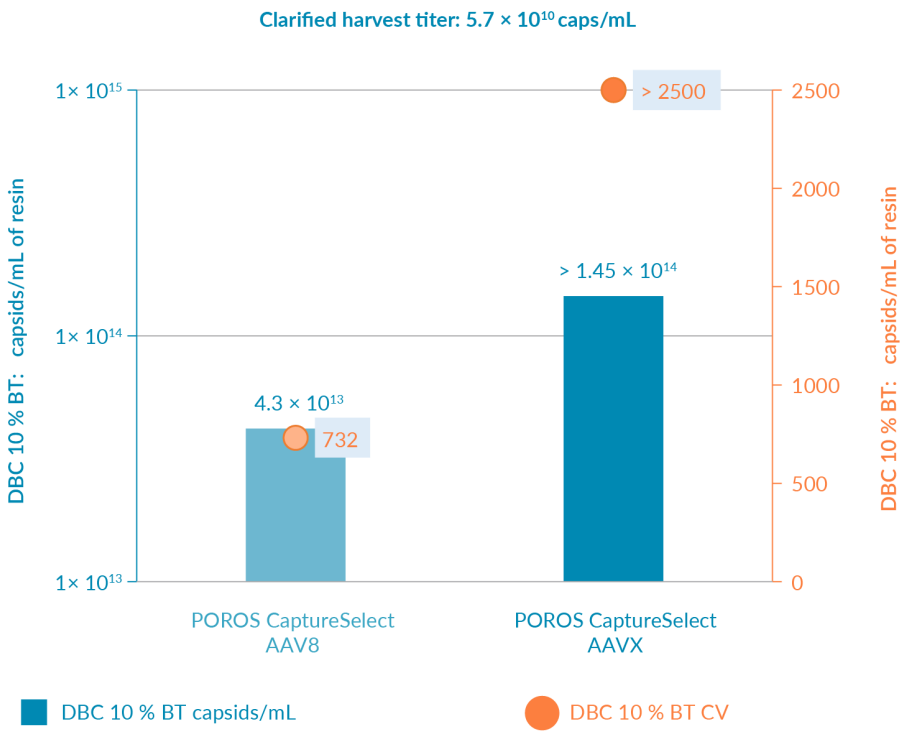
Binding capacity at 3 mins residence time and 10% breakthrough for POROS CaptureSelect AAV8 and POROS CaptureSelect AAVX at AAV titers of 4.3×10¹⁰ caps/mL (left) and 4.9×10¹¹ caps/mL (right).



The blue bars represent the binding capacity in terms of capsids per mL of resin at 10% breakthrough. The orange dots represent the column volumes that lead to 10% breakthrough.

FIGURE 4

Binding capacity at 1 min residence time and 10% breakthrough for POROS CaptureSelect AAV8 and POROS CaptureSelect AAVX at an AAV titer of 5.7×10¹⁰ caps/mL.



The blue bars represent the binding capacity in terms of capsids per mL of resin at 10% breakthrough. The orange dots represent the column volumes that lead to 10% breakthrough.

capacities compared with the 3 mins residence time. At both residence times, there was no breakthrough on AAVX, with loading volumes up to 2,500 column volumes (CV).

The results from these binding capacity studies led to three main conclusions:

1. The AAVX resin has a better AAV8 binding capacity than the AAV8 resin
2. Binding capacity increases with harvest titer
3. Residence time has no significant effect on the binding capacity

DEFINING OPERATING CONDITIONS FOR PURIFICATION OF AAV8 & AAV2

The operating conditions for the capture of the AAV8 serotype were defined according to the DBC data obtained previously. Screening of capture conditions was performed on 1 mL-pre-packed columns with AAV8 or AAVX resin **(Figure 5)**.

The material loaded onto the columns was a clarified supernatant containing AAV8 vectors. To align with our AAV manufacturing operating conditions, the maximum loading time selected was 18 hours – (overnight loading). Two residence times were evaluated: 3 mins and 1 min.

The loading volumes selected were 356 CV (for AAV8 and AAVX) with a 3 mins residence time, and 600 CV (AAV8) and 1080 CV (AAVX) with a 1 min residence time. These CVs are all below the resin binding capacities at 10% breakthrough defined earlier **(Figures 3 & 4)**.

After loading and washing, purified product was recovered during the elution step at low pH and was immediately neutralized.

DOI: 10.18609/cgti.2022.001

The clarified harvest and eluent were tested for viral genome (VG) titer.

Similar quantities of AAV vectors were loaded on the AAV8 and AAVX resins at 3 mins residence time. As shown in Figure 6, the quantity of AAV8 vector recovered after elution and the AAV8 yield was very similar for both resins. The resins showed no significant difference in performance when loading at 3 mins residence time or at 18 hours loading time.

Results at 1 min residence time are shown in Figure 7. As a consequence of the different binding capacities at 1 min residence time, the loading times were different for the two sorbents – 10 hours for the AAV8 resin and 18 hours for the AAVX resin. Thus, the total quantity of AAV8 capsid loaded on the resins was around 1.8 times higher for the AAVX resin compared with the AAV8 resin. As a result, the quantity of purified recovered product for AAVX was approximately 1.7 times higher. The step yields for both resins were also very similar and close to 90% which is higher than the yield of around 70% obtained with a residence time of 3 mins.

These results indicate that it will be possible to switch from POROS CaptureSelect AAV8 to AAVX for the purification of AAV8 serotype.

Based on these results with POROS CaptureSelect AAV8, the AAVX resin was also evaluated for the capture of another serotype of AAV: AAV2 (Figure 8). The aim was to compare POROS CaptureSelect AAVX with an affinity resin from another supplier, which is currently used at Yposkesi for AAV2 processes.

The screening of the capture conditions was performed on 1 mL prepacked columns. Two residence times were applied for the AAVX resin: 3 mins and 1 min. The residence time applied to the other affinity resin was 8 mins, according to supplier's recommendation.

Three purification conditions were screened for the capture of the AAV2 vector. At 3 mins residence time, the volume of clarified harvest loaded on the column was 455 CV, whereas at 1 min residence time the volume loaded on the column was 1,440 CV. The same starting material was used for all trials.

For the other resin, only 340 CV were loaded since the residence time applied was higher. After column washing, the product was eluted at low pH, and the loading and elution fractions were tested for VG titer.

Using the AAVX resin and decreasing the residence time from 3 min to 1 min resulted in an increase in VG yield from 57% to 89% (Figure 9). Using the affinity resin from another supplier with higher residence time

FIGURE 5

Experimental plan for the definition of operating conditions for purification of AAV8.

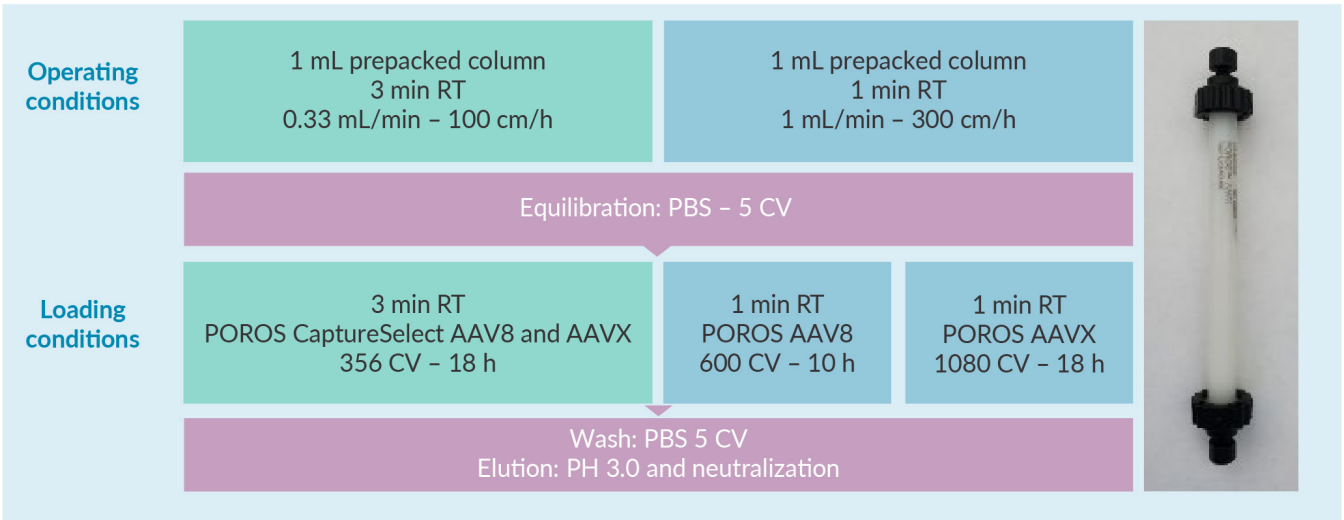
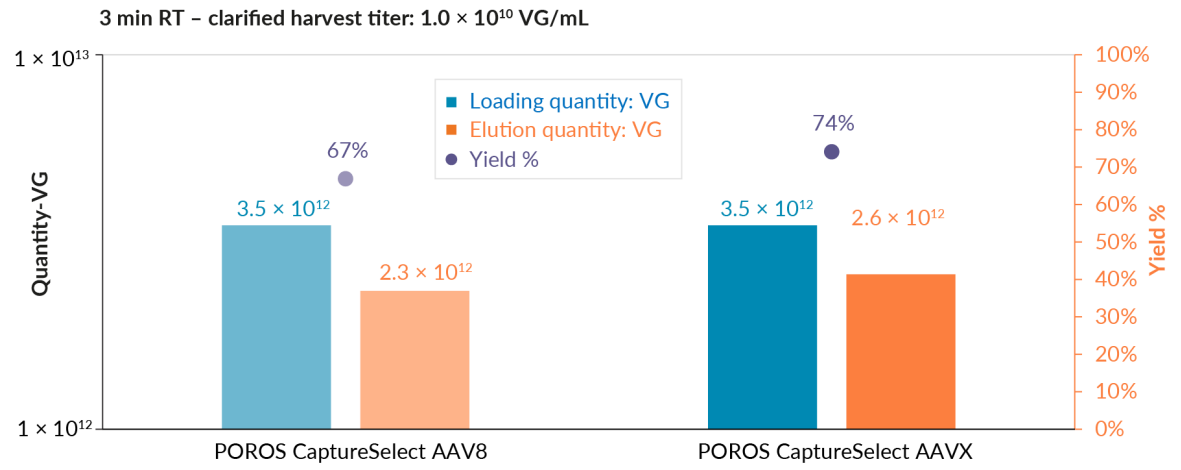


FIGURE 6

AAV8 capture conditions – results for 3 mins residence time.



The blue bars represent the product quantity loaded on each column. The orange bars represent the quantity of purified product recovered during the elution. The red dots represent yield.

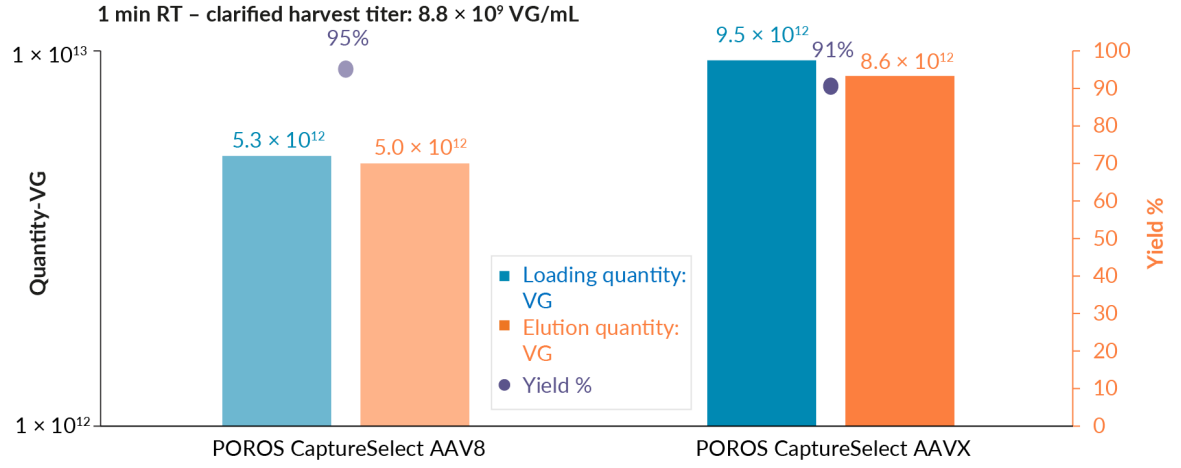
(8 mins, imposed because of the compressibility of the media, and as recommended by the supplier), resulted in a low volume loaded on this column. The AAV2 yield is significantly lower than the yield obtained with AAVX: 48% yield, versus 70–90% yield obtained with AAVX.

This part of the study demonstrated that using a lower residence time results in higher AAV binding capacities for both Thermo Fisher Scientific resins, and that the AAVX resin shows better results for the capture of AAV8 and AAV2 vectors. The volumes of clarified harvest that can be loaded on AAVX without any AAV breakthrough in the flowthrough are 1080 CV for AAV8, and 1440 CV for AAV2.

The promising results obtained with POROS CaptureSelect AAVX led us to select this resin for the next part of the study and to work with a residence time as close as possible to 1 min.

FIGURE 7

AAV8 capture conditions – results for 1 min residence time.



The blue bars represent the product quantity loaded on each column. The orange bars represent the quantity of purified product recovered during the elution. The red dots represent yield.

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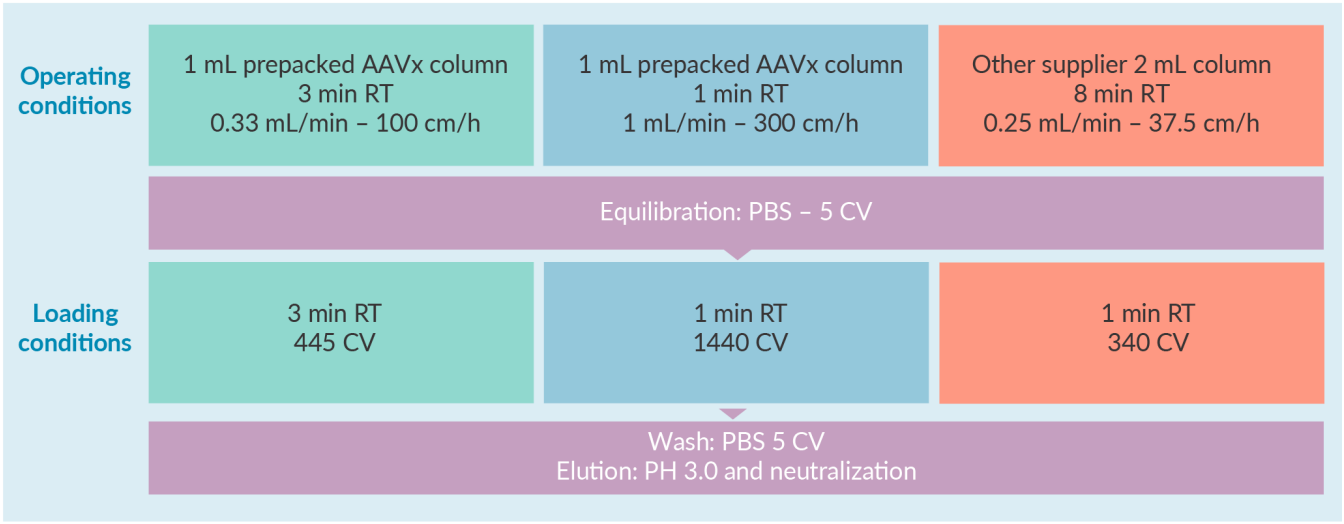
FIGURE 8

Experimental plan for the definition of operating conditions for purification of AAV2.



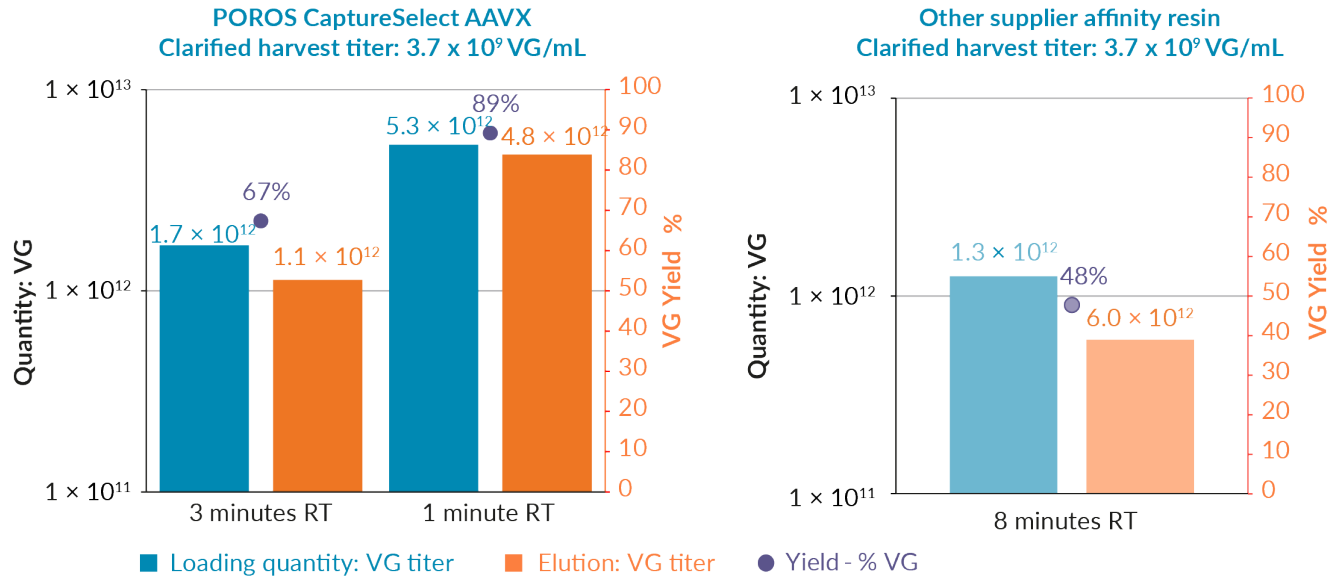
SCALE-UP OF THE CHROMATOGRAPHY STEP

The experimental conditions determined using AAVX for the capture of AAV2 and AAV8 vectors were adapted for the purification of clarified harvest from a 10-liter bioreactor (Figure 10).

The volume of resin necessary to purify a 10 L clarified harvest was calculated by applying the column loading capacity in terms of CV determined previously during the screening for AAV8 and AAV2 processes. This AAVX resin volume was found to be 14.3 mL for AAV8 capture and 11.3 mL for AAV2 purification process.

FIGURE 9

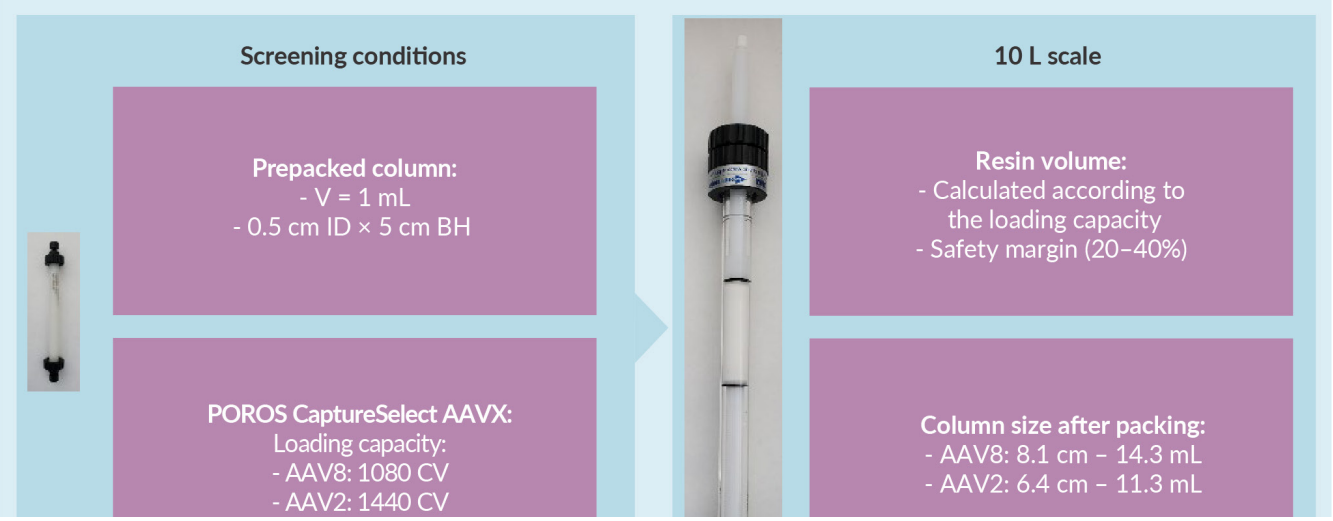
Definition of the operating conditions for the purification of AAV2 using POROS CaptureSelect AAVX (left) or alternative supplier's affinity resin (right).



The blue bars represent the product quantity loaded on each column. The orange bars represent the quantity of purified product recovered during the elution. The red dots represent yield.

FIGURE 10

POROS CaptureSelect AAVX chromatography scale up.



Conditions to be tested are listed for small-scale (left) and 10-liter scale (right).

AAVX resin was packed in a 15 mm internal diameter glass column, which allowed for a resin bed height that would be easily transferrable to GMP scale. The column bed height was 8.1 cm for AAV8 purification and 6.4 cm for AAV2 purification.

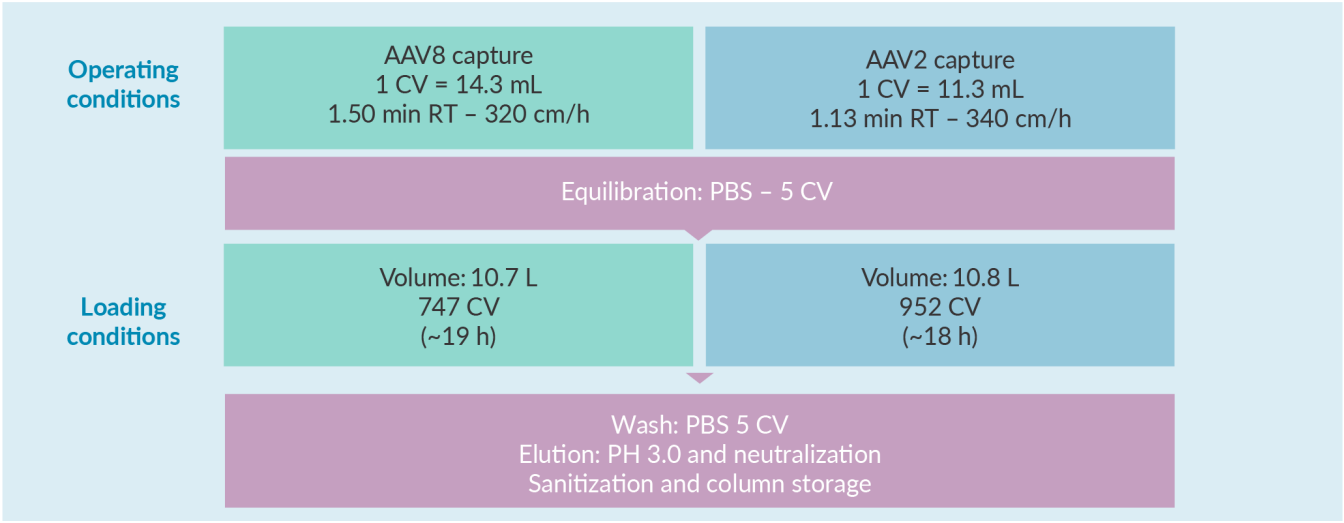
In order to obtain the starting material for resin evaluation, two 10 L bioreactors were used to produce AAV2 and AAV8 vectors from HEK cells.

After AAV production, cells were lysed, and the lysate was clarified and filtered using a 0.22 µm filter. After lysate filtration, the pool titer was 1.10 x 10^{11} VG/mL for AAV8 vectors and 3.70 x 10^{9} VG/mL for AAV2 vectors.

The selected operating conditions for the AAVX resin to purify AAV8 and AAV2 from a 10 L clarified harvest are shown in Figure 11.

FIGURE 11

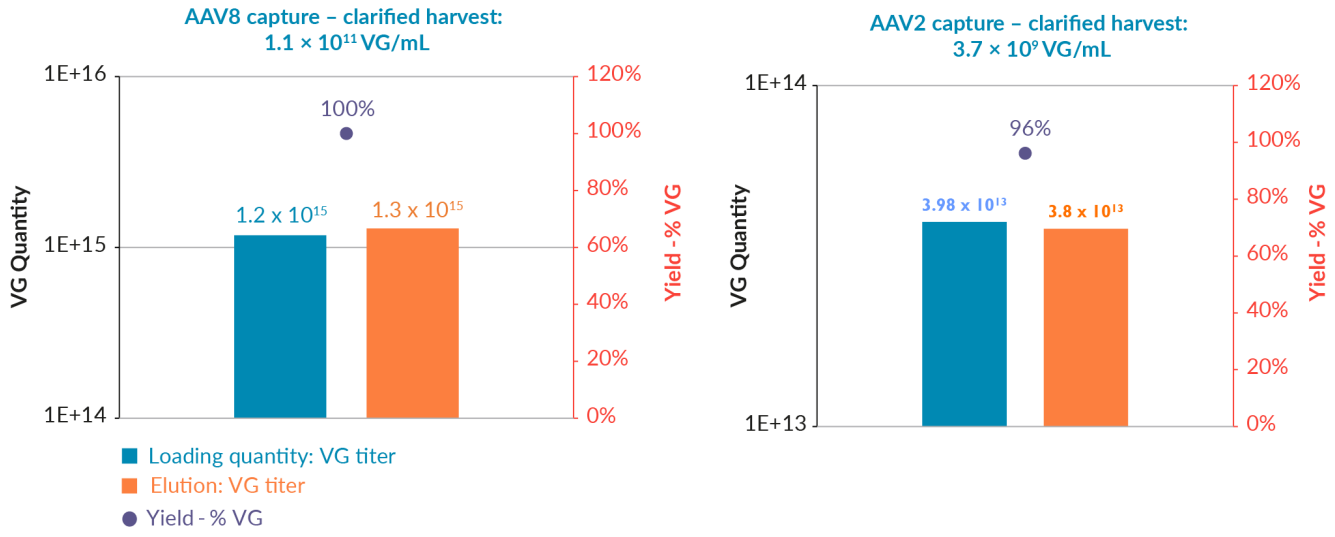
Selected chromatography operating conditions at larger scale.



DOI: 10.18609/cgti.2022.001

FIGURE 12

AAV capture on POROS CaptureSelect AAVX – yields for AAV8 (left) and AAV2 (right).



The blue bars represent the product quantity loaded on each column. The orange bars represent the quantity of purified product recovered during the elution. The red dots represent yield.

The residence time for both AAV processes was close to 1 min. 747 CV of clarified harvest were loaded on to the AAVX resin for AAV8, and 952 CV for AAV2, while the loading times were in the same range. The purified products were recovered during elution at low pH and then neutralized. VG titers, total protein content, and residual DNA levels were assayed in the clarified harvests (starting materials) and in the elution fractions.

The pressure was monitored at the inlet of the column during the loading step for the AAV8 and AAV2 capture process. The pressure slightly increased during the loading stage but stayed within an acceptable range. The pressure was around 1.5 bars at the end of the loading step, which helps to provide good conditions for a transfer to GMP scale.

Even though VG titers in the starting material were very different for the AAV2 and AAV8 serotypes, the final yields of the capture step are close to 100% for both serotypes and there was good scalability from lab scale development to the 10 L scale (Figure 12).

Additional experiments revealed that the purity of AAV vectors captured with AAVX resins appears to be very high. There was an impurity reduction of over 99% in the purified product after capture on AAVX for each serotype. This clearance rate could be even further optimized by adding an intermediate washing step or implementing a polishing column after the AAV capture step.

CONCLUSIONS

This long-term study with POROS CaptureSelect AAVX resin has highlighted several advantages of AAV capture using this resin compared to other affinity resins commercially available:

- Flexibility in terms of serotypes: capture of AAV1 to AAV9 serotypes and synthetic and recombinant serotypes
- Possibility to standardize a purification platform for several AAV serotypes with only a few adjustments
- Cost reduction due to shorter residence times and very high loading volumes.

- Low level of impurities captured on the resin. This could be further optimized for each serotype if needed (wash conditions screening or addition of a polishing step)
- Good scalability of the downstream platform. It is compliant for a large-scale GMP AAV manufacturing process

Overall, Yposkesi concluded that the POROS CaptureSelect AAVX resin appears to be a great tool to improve purification processes in terms of quality, cost, and standardization. Yposkesi plan to implement this resin for the purification of other AAV serotypes.

ASK THE EXPERTS

Nicolas Laroudie (Thermo Fisher Scientific) joins **Vincent Ravault** (Yposkesi) to answer readers' questions on implementing POROS CaptureSelect technology into viral vector production.

Q Can the POROS CaptureSelect resin be cleaned and re-used?

NL: Yes, the resin can be cleaned and reused. Many customers use the resin once, particularly CDMOs that deal with multiple serotypes and multiple transgenes and want to avoid spending a lot of time validating cleaning. But the resin can absolutely be cleaned and reused, and many customers are doing that.

Notably, the resin is not very caustic stable, and so for cleaning, we do recommend using acidic solutions such as phosphoric or citric acid. In case of very dirty resin, we advise additional cleaning with chaotropic agents, such as guanidine hydrochloride or urea.

I would encourage people who want to clean and re-use the resin to reach out to their local application specialist, who can help them develop a process for this.

Q Apart from AAV2 and AAV8, do you have experience with other AAV serotypes and POROS CaptureSelect AAVX?

DOI: 10.18609/cgti.2022.001

VR: The goal for Yposkesi now is to expand this platform to a broad range of AAV serotypes. With our experience of AAV2 and AAV8 serotypes, we know that the AAVX resin is a good solution to use as a purification platform.

Currently, we are working with AAV5, 6, and 9, and the results so far are promising. We also know that we can work with modified capsids.

Q Is the resin available in a pre-packed format?

NL: Yes, we do have pre-packed formats. We have 1 and 5 mL pre-packed formats available that are compliant with standard benchtop chromatographic systems. We also have robocolumns available, at 200 µL and 600 µL, for high-throughput screening.

Of course, the resin can be purchased as bulk material and our local Field Application Specialists are happy to support customers in packing the resins in their own columns, whatever the scale.

Q Which additional washing conditions would be suitable for host cell protein and host cell DNA reduction?

VR: Several washing conditions are interesting to assess. For example, you can add an extra washing step using high salt concentration. You can also wash your column with a low pH buffer in order to remove impurities from the column before recovery of AAV in the eluate.

If you decide to implement the second washing step you have to be careful that your washing condition won't affect the integrity of your capsid. Moreover, if the washing step pH is too close to the pH of the elution buffer, a significant quantity of capsids could be eluted during your washing step, and as a result, lower your AAV yield during the elution.

Q Is the resin GMP compliant?

NL: The resin is used in GMP manufacturing by many of our customers. While not manufactured in a cGMP process, the resin is produced under an ISO 13485 environment.

When you purchase the resin, you can request the regulatory support package, including documents regarding quality, stability, production, control method, and so on. Those documents are useful when you make a product and submit a dossier to a regulatory agency.

For each of our commercially available CaptureSelect resins, we developed an ELISA assay to monitor the level of ligand leakage over the purification process.

Q Vincent, why did you use two different analytical methods during this study – ELISA for dynamic binding capacity and viral genome titers at termination during your screening?

VR: During our DBC study, a lot of fractions were collected in the flowthrough at the outlet of the column in order to calculate 10% breakthrough for AAV vectors, so we needed to use a high throughput assay for the analysis of the first full fractions. The ELISA assay allowed us to test several samples in parallel and to get the results quickly, in around half a day.

In the screening study, the number of samples was much lower – only two samples for each set of conditions screened were produced – so here we used an internal assay for the quantification of the viral genome titer in the product. The viral genome titer was determined by qPCR for each serotype.

Q Which resin can be implemented for a polishing step?

VR: Several different resins can be implemented for this step. Commonly, an anion exchanger is implemented in order to reduce host cell protein and host cell DNA. Anion exchange also has the capability to separate empty and full AAV capsids, and some suppliers have developed resins specifically for the polishing step. For more information, you can contact chromatography resin suppliers.

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BIOGRAPHIES

Vincent Ravault

DSP Expert, Process Development and Industrialization Department, Yposkesi

Vincent Ravault is a technician within the Process Development and Industrialization department at YposKesi since 2018. He focuses on upstream and downstream process development and optimization for AAVs and Lentiviruses vectors to support and advance their large-scale production for gene therapies. He is also involved in the technical transfer of processes for viral vectors from pilot scale to manufacturing scale. Graduated of a Biochemistry degree, Vincent Ravault has over 15 years of experience at Pall Life Sciences where he was in charge of the technical support for chromatography resins. His main role at Pall was to provide purification solutions and strategies to customers. He was also involved in the promotion and evaluation of new products for downstream.

Nicolas Laroudie

Staff Scientist, Field Applications, Thermo Fisher Scientific

Biochemist by education, Nicolas Laroudie used to work for Généthon, France, between 2001 and 2011 as Head of Downstream Development. He was leading a team in charge of developing and scaling-up purification processes for AAV, retroviral and lentiviral vectors

DOI: 10.18609/cgti.2022.001

used in gene therapy treatments. He then joined Merck Millipore as a BioManufacturing Engineer where he used to technically support European customers for all DSP technologies - from clarification to sterile filtration, including TFF and systems - with a strong focus on chromatography. In particular, he took an active role in the establishment of a fully continuous, large-scale disposable DSP process for the purification of a monoclonal antibody, within the framework of a large multi-company European consortium. He eventually joined ThermoFisher Scientific in 2019 as Field Application Specialist for purification, technically supporting the implementation of POROS and CaptureSelect chromatography products for south-western European customers.

AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

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

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

ARTICLE & COPYRIGHT INFORMATION

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

Webinar recorded: *Oct 26 2021;* **Revised manuscript received:** *Jan 4 2021;* **Publication date:** *Jan 24 2022.*

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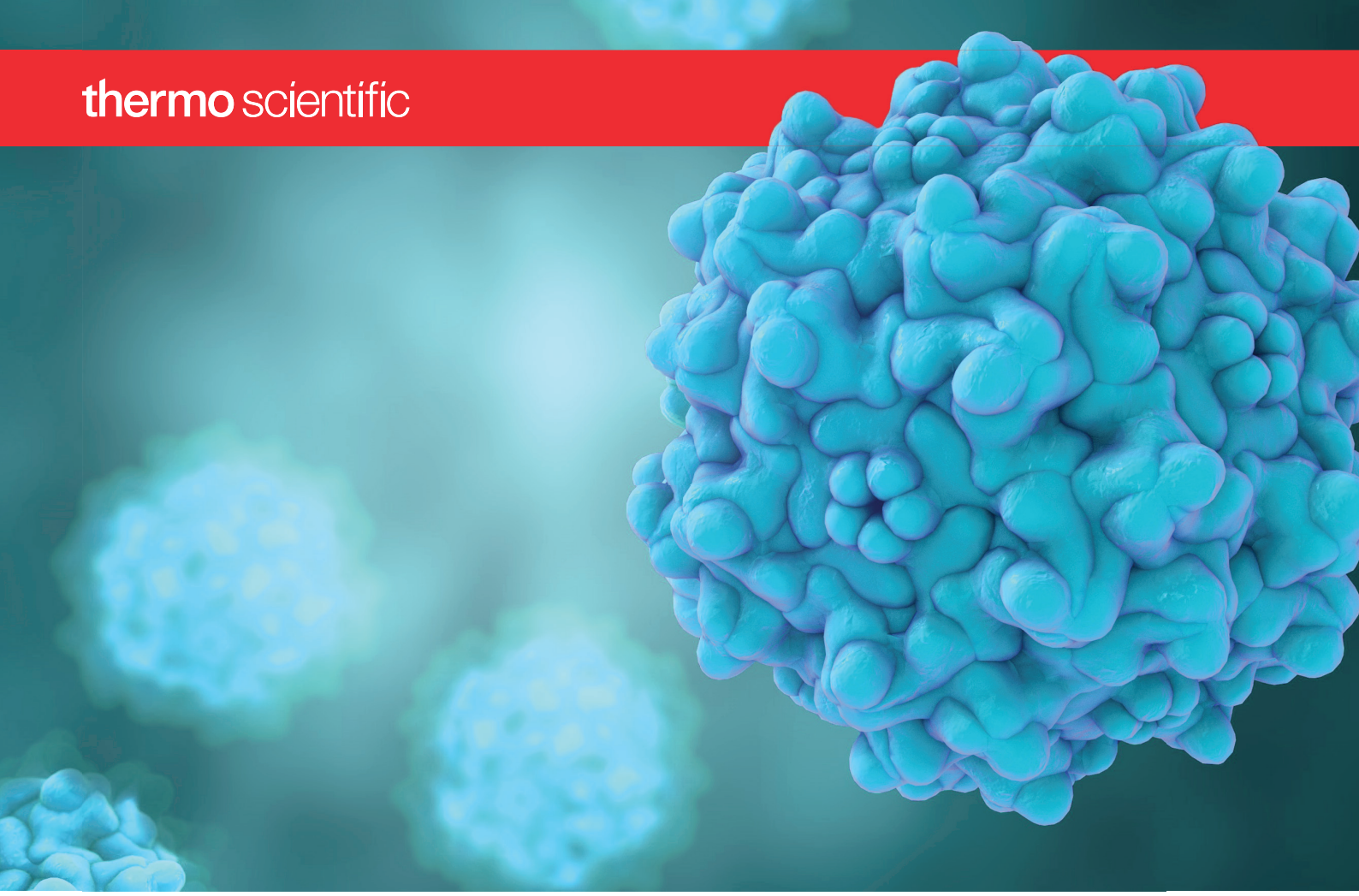

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ENHANCING VECTOR BIOPROCESS SCALABILITY

INTERVIEW

Tackling scalability challenges in AAV manufacture

KYLE GRANT is a trained PhD chemical engineer with a concentration in molecular virology. Kyle has extensive experience in the molecular analysis of virus host cell interactions, as well as innovation around commercial grade unit operations to support CMC activities for the application of viral vectors in gene therapy. This multi-scale approach allows for comprehensive insight into the development of methods for provision and release of novel drug candidates to address the unmet need for patients where there is no available cure.

Cell & Gene Therapy Insights 2022; 8(1), 25–28

DOI: 10.18609/cgti.2022.014

Q What are you working on right now?

KG: Manufacturing release specification development and manufacturing technology development.

Q You've mainly specialized in virus production throughout your career in academia & industry – can you frame for us the journey the field has taken over that period in terms of enhancing scalability?

KG: Largely, development of the field has been born out of Cost of Goods. The use of novel cell lines exists to relieve the burden of acquiring GMP grade reagents that can add to the cost of drugs for the patients as well as the company. By manufacturing novel reagents, one can increase the yields, increase the quality of the material, and take advantage of the elegance of the balance between viral replication and the host cell defense mechanisms therein.

Q What are the key scale-related challenges in AAV vector processing that you face on a day-to-day basis?

KG: The chief issues facing gene therapy manufacturing globally – particularly for biologic-based gene therapy manufacturing – are related to the supply chain. This is due to the paucity of plastics, bags, and now liquid reagents that are critical for the GMP manufacture of new drug classes or viral-based gene therapies.

On the other side, the innovation can pose an issue. The industry is trying to adapt an academic process to generate something at a commercial scale. In general, there are inefficiencies in how gene therapy companies approach manufacturing in terms of the unit operations. The industry often tries to use solutions from a different drug class – monoclonal antibodies, or biologics in general – that may not be appropriate for AAV or other viral vector systems. This approach may lead to safety or regulatory issues in the clinic, because the material being generated may have some quality and potency issues.

Q What is Voyager's approach to addressing these issues, & what tools and technologies are assisting you?

KG: One approach is to engage with vendors that are unique enough to address these issues. There are plenty of vendors that will provide start to finish services. You can even outsource to an external partner for commercial manufacture.

There is still a lot of innovation that could happen on the unit operations side, in particular surrounding bioreactor configurations. Mixing matters, details matter, and the idea of applying the same kind of brute force approach to HEK293 cells that you might use for another, hardier cell line

"The chief issues facing gene therapy manufacturing globally – particularly for biologic-based gene therapy manufacturing – are related to the supply chain."

DOI: 10.18609/cgti.2022.014

deserves more attention. There are gene therapy companies with unique bioreactor configurations that may address these considerations.

Q How have Voyager's platforms evolved over recent years, & what are the advantages of these platforms relating to scalability, specifically?

KG: There are exciting challenges for Voyager's manufacturing platform. Voyager had extensive experience in SF9 manufacturing for AAV and is considered in the industry as a leader in this technology. We have had some clinical experience using this manufacturing platform. We continue to explore this technology and its potential applications to our proprietary TRACER- generated capsids. These approaches represent opportunities for scalability for the manufacture of these novel capsids, which offers the potential for treating patients by infusion rather than neurosurgical approach.

The improvements in manufacturing may be advantageous because the Cost of Goods can be reduced. This means that cost can be passed on to patients and their families at a much lower rate. For instance, with more transient-based processes, Cost of Goods is more of a factor. In addition, the scalability is quite linear from a 2- to 20,000-L scale. The longevity of the platform for the life of the asset of the company means you do not need to generate new DNA every time you make a GMP run.

Q Tell us more about the current Voyager R&D pipeline.

KG: As of now, we are not a clinical-stage company. However, we are currently conducting research and development activities that are aimed at future submissions to the FDA seeking regulatory approval to treat patients in clinical studies.

Voyager is focused on diseases of the central nervous system and other areas. We have an active pipeline for a wide variety of indications. We believe we have a developing portfolio that aims to provide clinical approaches that are targeted to be best in class for these indications.

Q What are your own chief goals & priorities over the next few years?

KG: My personal goal is to embolden and strengthen Voyager's portfolio and

"The improvements in manufacturing may be advantageous because the Cost of Goods can be reduced. This means that cost can be passed on to patients and their families at a much lower rate."

strengthen our manufacturing capabilities. I also want to increase communication with industry leaders in gene therapy manufacturing. Working with the Voyager team, we want to continue to innovate all aspects of manufacturing, including cell line development, media development, and design of our engineering platform for providing rapid turnaround on pre-clinical candidates.

I am also looking forward to looking for ways to innovate downstream processing to safeguard against potential issues surrounding toxicity which have been reported in the gene therapy industry. This starts with making sure we have release specifications that can drill down into the fundamental characteristics of the drug.

Harmonizing the standards, potency assays and across the industry may help simplify and expedite clinical approval and ensure that these novel drugs reach patients with unmet medical needs without delay.

AFFILIATIONS

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AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

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

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

Article source: *Invited.*

Interview conducted: *Dec 12 2022;* ***Publication date:*** *Jan 27 2022.*

DOI: 10.18609/cgti.2022.014

CELL & GENE THERAPY INSIGHTS

February 2022

Business Insights

CELL & GENE THERAPY INSIGHTS

Business Insights

February 2022
Volume 8, Issue 1

CELL & GENE THERAPY INSIGHTS

BUSINESS INSIGHT: FEBRUARY 2022

COMMENTARY/OPINION

Advancing patient access to cell & gene therapies: partnerships, pilots, & psyche

Heather McDonald

Cell and gene therapies offer patients the hope of durable treatment with curative potential in as little as a single dose. Having demonstrated unprecedented efficacy in a number of diseases, including lifelong disorders previously deemed incurable, success of these transformative therapies heralds a new era of personalized medicines. While still in their infancy, cell and gene therapies are projected to expand to multiple disease areas within the next decade. Although showing tremendous promise, there are also challenges in ensuring that patients can access these new modalities. Payers, for example, have articulated concerns related to affordability and uncertainty around long-term clinical benefit. Manufacturers, meanwhile, face challenges navigating payer evaluation frameworks, given that cell and gene therapies are typically one-time treatments with a lifetime value proposition studied in comparatively small, sometimes single-arm clinical trials of a finite duration. Patient access for cell and gene therapies will therefore depend on collaborative efforts within the healthcare ecosystem and a willingness to share not only in the benefits, but also the risks associated with their reimbursement.

Cell & Gene Therapy Insights 2022; 8(1), 43–51

DOI: 10.18609/cgti.2022.017

TRANSFORMATIVE CELL & GENE THERAPIES IN AN ERA OF PERSONALIZED MEDICINE

For decades, many diseases have been labeled as 'intractable', meaning that medicine could not provide an answer for patients beyond, to a varying extent, addressing the symptoms they experience. However, scientific advances have led to the development of cell and gene therapies (CGTs) that bring transformative value, offering patients the hope of durable treatments with curative potential in as little as a single dose. Cell and gene therapies address the root cause of disease, developed with the intent of preventing, treating, and potentially even curing illnesses. This applies not only to rare genetic diseases, but also to more common diseases, such as certain immune disorders, cancers, and degenerative diseases. First-generation CGTs are already impacting the lives of patients around the world and hold the potential to dramatically alter the standard of care across multiple conditions, providing long-term benefit for patients.

REIMBURSING CELL & GENE THERAPIES: PAYER CONSIDERATIONS

Although CGTs hold tremendous potential, payers have articulated concerns about reimbursement of these new therapies under a backdrop of potential risks and uncertainties. First, despite benefits being realized over a patient's lifetime, payment for CGTs is potentially front-loaded due to their one-time administration [1]. This differs from the current healthcare system and drug reimbursement approach, which is built around chronic administration of and payment for treatments, and payers may consequently face affordability challenges within the context of an annual drug budget. Second, clinical development programs for CGTs tend to differ from those adopted for previous treatment modalities. Whereas randomized controlled trials have long been considered the gold standard for evidence generation, this approach poses several challenges for the clinical evaluation of many CGTs due to small patient populations, the absence of therapeutic alternatives for patients, ethical considerations regarding clinical equipoise vs. standard of care, and potentially the use of endpoints that have yet to be validated in new therapeutic areas but which capture critical benefits conferred by these new treatments [2]. Furthermore, most CGTs have limited long-term efficacy data relative to their long-term value proposition. Consequently, the evidence packages for CGTs tend not to fit within the existing health technology assessment (HTA) frameworks that are used to evaluate a product's value for money. As a result, payers foresee risk in terms of whether a CGT will be as effective and durable as proposed in its value proposition (i.e., whether the proposed value for money will be realized).

COMMERCIALIZING CGTS: MANUFACTURER CONSIDERATIONS

While payers foresee risks in reimbursing CGTs, manufacturers bear risk in the development, manufacturing, and overall commercial viability of bringing these treatments to patients. The manufacturing of CGTs is differentially costly and complex relative to other modalities. A recent article by Micklus [3] notes that.

> "unlike small molecules and even some biologics, cell and gene therapies have a more challenging road to commercialization and success for many reasons..."

With many CGTs, manufacturers are producing a highly complex product that is delivered on a personalized basis to each patient. As noted by Moutsatsou, "in contrast to traditional biopharmaceutical production where processes are based on one, well-characterized strain and can be repeated relatively well, cell

DOI: 10.18609/cgti.2022.017

and gene therapy manufacturing processes require increasingly adaptive process strategies that take the inherent variability of the living product into account" [4]. Furthermore, CGT technologies and products are very diverse, meaning that there are challenges unique to each treatment and that there is no 'one size fits all' approach to chemistry, manufacturing, and controls (CMC). For example, depending on the technology in question, manufacturers must consider additional manufacturing challenges such as genetic stability and tumorigenicity, immunogenicity and off-target effects, amongst others [5]. Manufacturers must therefore invest heavily in learning, refining, and optimizing CMC processes to ensure patient safety from preclinical research through to early usage and ultimately full-scale commercialization. Moreover, for those CGTs that are truly personalized treatments, the investment must be made many times over for each individual asset.

Regarding delivery and administration, CGTs require careful patient selection, as well as multiple detailed preparation and planning steps to ensure optimal treatment. For example, stem cell-based therapies for neurological disorders, such as Parkinson's Disease, are very complex procedures involving not only a carefully produced and characterized product, but also tailor-made delivery devices and highly specialized neurosurgeons capable of administering the treatment. Administration of the final product into a patient's brain may require sophisticated live imaging, as well as customized delivery devices that enable precise injection of therapeutic material. Finally, post-administration, patients need to be monitored for years to allow both the detection of the clinical benefit as well as any potential safety events.

In sum, the manufacturing, delivery, and administration of CGTs are highly complex and costly, requiring significant investments in materials, processes, manufacturing technologies, and skilled personnel. All of these components are important in ensuring patient safety and commercial scalability. Notably, this investment is paired with significant uncertainty about the commercial uptake of these new modalities. Presently, commercial uptake is highly uncertain due in part to the already-described mismatch between existing HTA frameworks/drug reimbursement models and the clinical trial designs typically employed for CGTs (i.e., comparatively small, sometimes single-arm trials that observe benefit over a shorter period of time than the duration of benefit suggested in their value proposition) [6]. If this gap leads to delayed access, reduced access, or denial of access, manufacturers will face challenges in the commercial predictability, stability and overall viability of bringing new treatments to market. Underscoring this risk are products such as ChondroCelect®, Glybera and Zytenglo™, all of which were removed from the European market due to commercial viability challenges [7–9]. Without a clear and viable commercial path moving forward, investment in developing future life-altering CGTs will dwindle.

EARLY SUCCESSES IN DELIVERING PATIENT ACCESS

Initiatives have been established by some regulatory and HTA organizations to either explicitly recognize the nuances of CGTs or to create pathways for innovative therapies that treat rare diseases and/or address a significant unmet medical need. These include, for example, the Food and Drug Administration's Regenerative Medicine Advanced Therapy (RMAT) designation, the European Medicines Agency's (EMA) PRIME designation, the EMA Committee for Advanced Therapies, the United Kingdom's Innovative Licensing and Access Pathway (ILAP) and the National Institute for Health and Care Excellence's (NICE) Highly Specialized Technologies Guidance [6,10–13]. While these will continue to be important evolutions for CGT commercialization, they will need to be paired with solutions at the pricing and reimbursement level to ensure that efforts made

in regulatory and HTA approaches can be converted into meaningful market access for patients. The final step in this pathway is the focus of my discussion below.

Despite the payer and manufacturer challenges already described, there are some early successes with reimbursement of CGTs. Reasons for these early successes include relatively small patient populations (which mitigate budget impact concerns), the high level of perceived innovation and therapeutic value for patients, and innovative funding solutions that aim to address some of the uncertainty and affordability challenges expressed by payers [14,15]. Some examples of the innovative approaches taken to date are outlined below.

Outcomes-based models share risk between payers and manufacturers by making payment contingent on pre-specified outcomes, either via milestone payments, or by use of rebates that can be applied if targets are not met or if supplemental therapy is needed. These models may be modified to be dependent on a specific outcome, a duration of effect, or magnitude of net benefit. Outcomes-based pricing has already been implemented for a number of CGTs, with agreements reached or under consideration for Zolgensma®, Luxturna®, and some chimeric antigen receptor T-cell (CAR-T) therapies in certain countries. For Zolgensma®, a gene therapy product for spinal muscular atrophy, AveXis/Novartis proposed a 5-year outcomes-based agreement with novel pay-over-time options [16]. In the case of Luxturna®, a gene therapy for treatment of inherited blindness, Spark Therapeutics offers outcomes-based rebates and an innovative contracting model as part of a shared risk arrangement, linking payment to both short-term efficacy and longer-term durability according to predefined outcomes [17]. For CAR-T therapies, Kymriah® and Yescarta®, a variety of outcomes-based models have been used across Europe, including outcomes-based rebates in Germany or outcomes-based staged payments in Italy and Spain, with predefined outcomes including survival and/or response rates [18]. In France and the UK, coverage includes a requirement for evidence development, including longer-term follow-up and post-launch data from treated patients [18].

Models that address uncertainty as it relates to affordability have also emerged and either have or can be applied for CGTs. These include patient or expenditure thresholds, annuity models wherein the payment for a treatment is spread over time, so-called 'Netflix models' wherein payers pay a fixed price per year to treat all patients, and insurance offerings such as the Cigna Embarc Benefit Protection program wherein US payers can provide coverage for Zolgensma and Luxturna by increasing insurance premiums for all plan members by roughly $1 per member per month, with no additional out-of-pocket costs for patients receiving the therapy [19,20]. Recently, Aetna announced the launch in the US of a designated Gene-based, Cellular, and Other Innovative Therapies (GCIT) network designed to enable access to new therapies while helping to manage their costs [21].

PREPARING FOR THE FUTURE: PARTNERSHIPS, PILOTS & PSYCHE

The solutions we have seen to date involve approaches that can work within the current ecosystem, using rationally developed modifications to conceptual frameworks and infrastructure. While this has the benefit of delivering immediate solutions for the products at-hand, it does not solve for the overall transformation that lies ahead, which will include products intended for larger populations and expansion of products into additional indications. We must collectively plan for a world wherein 10–20 new CGTs may be approved yearly in the US alone by 2025 [22].

But how do we get there? How can we make decisions and choices today that optimize for the ecosystem of the future? How do we work towards a future state that allows for sharing of both the risks and the benefits that CGTs bring for payers, healthcare systems, and societies, while also sufficiently rewarding innovation such that manufacturers can

DOI: 10.18609/cgti.2022.017

continue to bring new generations of solutions for patients?

1. Acknowledge & share risks & benefits

Within the healthcare ecosystem, many stakeholders are involved in helping patients access CGTs including healthcare professionals, healthcare institutions and delivery networks, medical thought leaders, payers, and caregivers, amongst others. Within this context, there are substantive patient access challenges to consider in terms of readying healthcare system infrastructures for the clinical adoption of these new modalities. However, for the purposes of this discussion, which is aimed at addressing pricing and reimbursement challenges, I simplify by focusing on three groups: patients, payers, and manufacturers. Within the patient/payer/manufacturer ecosystem, each group faces challenges and benefits. Though not exhaustive, the challenges and benefits for each group are listed in Table 1. The key to building sustainable solutions is a shared recognition not only of the challenges each group faces but also of the benefits. Furthermore, we need willingness amongst parties to jointly share risks such that each party can also sustainably realize respective benefits.

2. Collaborate, co-create & pilot

There are various ways to set up pricing and reimbursement solutions that address the needs of patients, payers and manufacturers. The optimal solution will depend on the disease area and treatment in question, the country/region/healthcare system in question, and the specific risks or uncertainties borne by each stakeholder in a given system. There is complexity and heterogeneity, to be sure. However, this should not deter us from tackling the issues. In order to learn and evolve, we must look to opportunities to create solutions together. In order to learn as quickly as possible, we must look for opportunities to pilot. Pilots could be run in specific settings or regions, could run for defined windows of time, and could be designed to address one or many facets of risk. Important in this approach is the openness to co-create and the ability to test new ideas, embrace the learnings, and apply these learnings to the next iteration or circumstance. In order to learn from pilots and evolve, we will also need to have open communication across parties, a willingness to change, and shared understanding of potential risks and benefits, such that no party is penalized if goals are not attained. We are unlikely to solve all challenges in one shot. However, we can move incrementally towards new solutions if we take specific steps with specific objectives in mind.

Setting an ecosystem up for success in a transformative area such as CGTs also means partnering across industry. Working collaboratively and cohesively across manufacturers will enable development of solutions that are implementable, effective, and sustainable across disease areas and for multiple stakeholders. In doing so, we can leverage our collective skills and expertise in order to create solutions that will allow patients to access CGTs now and for years to come.

3. Advance mindset, shape cultures, & leverage talent

Solutions to the CGT pricing and reimbursement challenge lie not only in sharing risk and co-creation, but also in our collective mindset and approach. Can we get to the table for an open discussion on where benefits lie, where risks are assumed, and how best to balance these within the ecosystem? Can we adopt a mindset wherein specific pilots may address some but not all challenges, but will contribute to our collective learning and problem-solving? And finally, can we collaborate to share key learnings and insights in order to advance patient access to CGTs in a sustainable way that minimizes future uncertainty? Farnia *et al.* for example, call for a societal commitment to an ongoing learning

TABLE 1

Benefits, risks, and commitments in the CGT reimbursement ecosystem.*

	Patients	Payers	Manufacturers
Key Role/ Impact	▶ Recipient of CGT	▶ Reimbursement of CGT	▶ Development, manufacturing and commercialization of CGT
Potential Benefits	▶ Health gains conferred by CGT (disease cure, reversal, or other as defined by value proposition) ▶ Reimbursement/funding of treatment in question ▶ Potential benefit from broader impact of treatment on caregiver burden ▶ Potential benefit from work productivity resulting in personal gain ▶ Less time engaging with healthcare system for a chronic health problem	▶ Opportunity to bring transformative medical solutions to covered patients ▶ Increase in health of reimbursed population ▶ Increase in societal health depending on value proposition (e.g. patient, caregivers, other) ▶ Healthcare system efficiencies (e.g., cost offsets) afforded by 1-time treatments ▶ Potential to benefit from future innovations	▶ Opportunity to bring transformative medical solutions to patients, healthcare systems and societies ▶ Revenue/profit ▶ Opportunity to develop future innovations
Potential Challenges	▶ Treatment with a therapy/modality for which healthcare system has relatively less experience ▶ Potential need for regular follow-ups, depending on post-treatment protocol and/or value-based payment models ▶ Potential to incur some drug costs (e.g., deductibles, cost sharing), depending on particular drug reimbursement plan ▶ Uncertainty regarding durability of value proposition	▶ Uncertainty re: value proposition ▶ Uncertainty re: durability of value proposition ▶ Affordability ▶ Expenditure predictability	▶ Costs of research, development, delivery ▶ High level of investment required to ensure patient safety across all steps in the manufacturing and delivery chain ▶ Uncertainty in commercial uptake given differences between current HTA/ payer decision-making frameworks and the way in which CGTs are studied and administered ▶ Demonstration of long-term value within the confines of clinical trial development
What is needed	▶ Willingness to attend follow-up appointments and share data for purposes of value assessment and ongoing evidence generation ▶ Willingness to adhere to clinical protocols that may be required for innovative pricing and contracting solutions	▶ Willingness to engage in discussions with industry re: new and sustainable approaches ▶ Willingness to partner in sharing risk ▶ View CGT reimbursement not just as a cost but as an investment in societal health/ welfare ▶ Willingness to share information as further evidence is generated via pilots or other value assessment initiatives	▶ Pricing and contracting approaches that share risk ▶ Mindset of partnering in long-term Healthcare system sustainability ▶ Willingness to share information and knowledge as further evidence is generated on assets and from pilots

*Examples for illustrative purposes, not an exhaustive list. Exact benefits and challenges may depend on the specific treatment in question.
CGT: Cell and gene therapy; HTA: Health technology assessment.

system wherein collaboration includes building systems to collect and analyze information regarding outcomes of interest for all stakeholders and where information addressing key areas of uncertainty is shared by and across involved parties [14].

For payers and healthcare systems, there must be a recognition that the benefits of CGTs are realized not only by the patients who receive them and the manufacturers who sell them, but also by healthcare systems and societies as a whole. Upfront investments in healthcare can yield very positive welfare benefits in the long run. Indeed, at the recent B20 Summit, representatives of the Business Federations and companies of the G20 issued policy recommendations advocating for countries to "treat healthcare as a strategic asset in which to invest, optimizing its return, sustaining the shift from volume to value-based healthcare, and focusing on holistic outcomes and net value generated for patients" [23]. Importantly, the authors of the policy recommendations noted that framing health as an investment does not translate to simply increasing expenditures on healthcare, but rather to enabling the promotion of a long-term vision for a sustainable healthcare system through more efficient and better targeted spending, with the ultimate goal of improving patient outcomes [23].

For manufacturers, talent and culture will be key. From a talent perspective, we need individuals who have a deep understanding of payer and healthcare system needs and an ability to clearly describe this perspective internally. We need agility, a willingness to partner and try something different even when there is ambiguity, and a thrust for embracing new approaches. We must enable creative thinking and empower teams to co-create and test solutions with our ecosystem partners. Finally, we must strive for solutions that deliver access to life-changing treatments, while also balancing healthcare system sustainability.

For patients, the ecosystem will rely on their willingness to engage both before and after receipt of treatment. While the specific experiences vary depending on the disease and treatment, the CGT patient journey can be complicated and time-consuming. As noted previously, a variety of stakeholders play an active role in delivering a given CGT to a given patient, and patients must navigate what can be a complicated pathway from diagnosis through to treatment. Time investments from patients and caregivers may be required for travel to appointments, referrals to specialists, wait times in clinics, and time in a medical centre for delivery of the CGT in question. In addition, patients and caregivers need to absorb and interpret vast amounts of information related to their disease and potential treatments in what are often new and evolving therapeutic spaces. Finally, patients will need to participate in long-term follow-up appointments and agree to having their clinical data captured and shared for the purposes of safety, effectiveness, and value for money assessments. This engagement with the healthcare system, both at the outset and through to treatment and follow-up, will be key for continued reimbursement for a given treatment (depending on the contracting/ funding agreement in place), will contribute to the larger body of evidence for a given

product, and will also help to advance payer and manufacturer's knowledge regarding how best to address the pricing, reimbursement, and patient access challenges inherent to CGTs.

BAYER'S APPROACH TO CGT DEVELOPMENT

At Bayer, we aspire to become an industry champion in the CGT space, delivering transformative therapies to patients who are in urgent need of care. Our strategy goes well beyond single investments or individual assets. Instead, we invest holistically in technology platforms. Our current focus is on establishing the following four platforms: (1) Induced pluripotent stems cell therapy (iPSC); (2) Adeno-associated virus (AAV) gene therapy; (3) Oncology cell therapies; (4) Gene editing. At present, our development portfolio comprises a number of candidates in various stages of clinical development, covering several therapeutic areas with high unmet medical need, with leading programs in Parkinson's disease, Pompe disease, hemophilia A, and congestive heart failure. Our approach looks at CGT assets holistically, rigorously planning the entire value chain from research and development (R&D) to manufacturing, commercialization, and patient access.

We are committed to our ambition of "Health for All" and believe that patient access to CGTs is essential. Bringing these products to patients will not only require adaptations in R&D, production, and distribution but will also command healthcare ecosystem solutions that accommodate new treatment modalities. We believe that collaboration, talent, and culture will be instrumental in the success of our CGT strategy and are therefore investing in research, advanced production capabilities, and talent development to ensure a successful translation of scientific ideas into broadly available treatments around the globe.

CONCLUSION

With their curative potential, CGTs are poised to fundamentally transform patient lives, healthcare systems, and societies for the better. However, given the challenges faced by payers and manufacturers, a paradigm shift is needed to enable broad and sustained patient access to these therapies. This shift is multidirectional, requiring stakeholders to partner in sharing both risks and benefits so that individuals, healthcare systems, and societies as a whole can realize the potentially transformative benefits of CGTs. With the emergence of new therapies on the market, there is an opportunity to learn as we go, embarking on pilots, partnerships, and other forms of collaboration to solve for the challenges that lie ahead.

We, at Bayer, are committed to developing partnerships and collaborations with our healthcare ecosystem stakeholders, including policymakers, payers, other manufacturers, and patients. In doing so, we believe we can co-create solutions that enable adoption of the expanding classes of CGT assets projected to be approved each year, ensuring that no patient is left behind.

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DOI: 10.18609/cgti.2022.017

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

Acknowledgements: *Editorial assistance was provided by Ingrid Koo, PhD, of FleishmanHillard, and funded by Bayer Inc. Critical review and input for content revisions provided by Jonathan M. Weber, PhD, MBA, of Bayer Inc.*

Disclosure and potential conflicts of interest: *The author is an employee of Bayer Inc. The author declares no other conflicts of interest.*

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

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *Nov 25 2021;* ***Revised manuscript received:*** *Jan 25 2022;* ***Publication date:*** *Feb 2 2022.*

CELL & GENE THERAPY INSIGHTS

BUSINESS INSIGHT: FEBRUARY 2022

INTERVIEW

Building from the ground up: business culture learnings for cell and gene therapy biotechs

JAMES NOBLE (JN) has served as a Non-Executive Director since September 2019. He formerly served as our full-time Chief Executive Officer since March 2014, and part-time CEO from July 2008 to March 2014, and is one of our co-founders. From July 2008 until March 2014, Mr Noble was also CEO of Immunocore. Mr Noble has over 30 years of experience in the biotech industry. He has held numerous non-executive director positions. Mr Noble previously served as Deputy Chairman of GW Pharmaceuticals plc and as a director of CuraGen Corporation, PowderJect Pharmaceuticals plc, Oxford GlycoSciences plc, MediGene AG and Advanced Medical Solutions plc. Mr Noble is Chairman of Orexo AB and also serves as Chairman of Sutura Therapeutics and as a director of Celleron Therapeutics.Mr Noble qualified as a chartered accountant with Price Waterhouse and spent seven years at the investment bank Kleinwort Benson Limited, where he became a director in 1990. He then joined British Biotech plc as Chief Financial Officer from 1990 to 1997. Mr Noble was previously Chief Executive Officer of Avidex Limited, a privately held biotechnology company that was our predecessor, from 2000 to 2006. Mr Noble holds an MA degree from the University of Oxford.

Cell & Gene Therapy Insights 2022; 8(1), 15–22

DOI: 10.18609/cgti.2022.016

Q Where would you place enterprise management in the list of biotech business/organization priorities as the field continues to mature, and why?

JN: Enterprise management evolves with the company. All data management is really about communication.

When you first start, the decision-making process and communication are the same thing. When we started Avidex, the forerunner of Adaptimmune, the whole company used to meet in a single room. We could decide what to do and realize why we wanted to do it, and everyone knew.

As a company evolves, it does so in a rather peculiar way. For one thing, you must become more formal about data management. In our case, we started as a research organization and for us, the real purpose of data management was not to give data out to anyone, but to exemplify the patents and to prove to people that we had done what we said we would do.

It started off with the basics, which in those days were unbelievably manual lab notebooks, with microfiches sent off to ex-munitions tunnels from the second world war to protect them forever. You had to import your information into the US for it to count there, so you solemnly sent off packages to a US-based lawyer. In other words, it was not very electronic. And there was no need for communication because everyone knew each other.

Data management evolves in several different directions. First of all, you reach the stage where not everybody is in the meetings anymore, so you have to have some sort of centralized system. However, the real problems occur when you move into development, because there are completely separate considerations to do with manufacturing and monitoring of third parties. Once you get into the clinic, there is a ton of information that you are not allowed to give back to people. You must also have records for a regulator as you go further down the line. For example, in manufacturing, you have to be able to prove everything you did. When we started, the manufacturing batch record for a cell therapy was 500 pages for a single patient, so it is a massive management exercise. Furthermore, manufacturing is of limited interest to the research people, let alone the finance people, so you then end up with data silos. You must work out what needs to be integrated, and what people do and do not know.

I cannot tell you how complicated it is. But you have to get it right – if you don't, you will fail either at one end with the patent exemplification, or at the regulatory end. As we are discovering with cell therapy, there are also biological surprises when working with patients. I am not a scientist, but the reality is clear that the biology is still in its infancy.

When we first set up in the US, we came across an additional problem in that the people in development and manufacturing tended to come from different big companies

"All data management is really about communication. When you first start, the decision-making process and communication are the same thing."

DOI: 10.18609/cgti.2022.016

where they were used to very clear but disparate sets of rules and architectures. Consequently, we had a lot of differing entrenched opinions about how things should be done. Trying to reconcile that both for research and at the same time, manufacturing, proved to be difficult.

The key for cell therapy in the future will be to figure out in which patient it is working and why, which means leveraging translational data. You need translational data to be fed back to the research people, so they can work on what is needed. If you do not have the data, you do not have a company, because you will not be able to persuade a regulator that you have done things properly. You will not know what has happened if something goes wrong, and you will be unable to defend your patents.

So the data architecture evolves with the company, and it must continue evolving.

Q Enterprise management is an expensive and time–consuming initiative to undertake, especially for biotechs with limited resources – what are the keys to pursuing it whilst remaining nimble and also not breaking the bank?

JN: It is much more expensive to not put it in place and then impose it retrospectively. I once was involved in a company that had to do so because it made a lot of acquisitions, each with a completely different architecture. That was going to take 10 years and cost a gigantic amount of money.

The key is to ensure the head of IT is not a technical person in the sense of understanding how the computer works physically, but that they understand the whole concept of IT strategy. Find a group of people who are technically literate but who do not want to spend their time setting up networks – they just want to spend their time on setting up architecture.

When we went to the US, we specifically hired Charles Wilfong, who is a strategic IT thinker. I think he would probably faint if you asked him to mend a PC, but what he does do well is working out which things should be integrated and which things should not, then setting up the right architecture for that to happen.

Q Looking back on your years as CEO of Adaptimmune, what would you pick out as the most important aspects of the culture you sought to develop within the company?

JN: The most important thing is honesty and trust. If you don't have that, everything else falls to bits. The three most important words are "I don't know".

You also need to have a no-blame culture. Every company makes mistakes. Every individual makes mistakes. The key is to work out how best to recover from them. In biotechnology, you are always going to have problems and errors. Sometimes experiments do not pan out. Academics can also cause difficulties as they can focus too much on publications. They do not work together in the same way as in a company. It is a different way of working because their career path depends on publications, so they do not like to share data. Honesty, truth,

sharing, and realizing you are part of a great enterprise are the most important cultural things.

Another way companies go wrong is by not speaking English anymore. They talk in jargon, acronyms, and "HR speak". Every person who comes into the room has got their own enjoyments in life, whether it is television, cycling, judo, or their children. They are human beings with their own life, and that is how you have to treat them. So you should talk straight English to them: that's the language they use at home – why should it change when they get to work?

We used to have new-starter lunches so that everyone met us. Anyone could say anything to anybody. We actively tried to instill that culture. The bigger a company gets the harder that is to maintain, of course, but nevertheless, it is the way to do it.

Q What for you are the most important ways in which cell/gene therapy biotech business leaders and their organizations need to continue to evolve to meet the future demands of the marketplace?

JN: One way or another, cell therapy is going to have to become more like a normal drug. For T cell receptor (TCR) therapies, that involves getting away from the requirement for tissue typing for HLAs.

It is difficult to explain to people that they are only eligible for the treatment if they have a certain tissue type and present a certain antigen, and that's not even considering the many other eligibility criteria. So the field is working on autologous therapies, as there is high unmet needs, while moving more towards allogeneic cell products because you have to end up with something that a big chunk of the population will be eligible to receive, and to receive quickly.

It is a long way away, but it would be better if we could address the toxicity of the preconditioning regimens. When you look at the toxicity of any cell therapy, 100% of patients have something wrong because they are getting chemotherapy. It also delays the cell therapy treatment, and it is still unclear as to whether some patients react badly to that. It is important to try to ameliorate the preconditioning, so a doctor can say "here it is, and you can go and get your cell therapy tomorrow morning".

It is about trying to make cell therapy less noticeable to the patient, essentially. Patients do not complain much about the preconditioning regimen infusion itself – I recall one patient saying it was a walk in the park compared to the chemotherapy she'd had before because it is a preconditioning regimen, not a therapeutic regimen. And the cell therapy infusion itself, for most patients, is amazing because it is just a 15-minute one-off. So some aspects of cell therapy are there already, but it must become more like a normal drug.

That is the way cell therapy is eventually going to go. People are going to work out how to make these things and deliver them themselves. Eventually, I do not think there will be much difference between a cell therapy and any other form of drug. Indeed, if it doesn't go that way, then I don't think we will see cell therapy fulfilling its potential.

DOI: 10.18609/cgti.2022.016

Q Focusing on the cellular cancer immunotherapy field in particular, what business model and technology trends do you expect to see developing over the short-mid-terms, specifically?

JN: Almost all cellular immunotherapy cancer regimes started in academia. If you look at why Carl June and Steve Rosenberg are so well known, it was because they were really pioneering things. They had to be very bold and experimental. These things could never have been done by a company. They had to be done in a research environment. For example, one of the issues with cell therapy is it is impossible to do toxicology studies in animals – even a chimpanzee doesn't have the same T cell receptor as a human. So you cannot do a lot of the studies that you would normally do. And the regulators accepted that fact.

We have spent years developing things in-house because the manufacturing processes development had to be approached as research programs, essentially. When we started, the viral vector was unbelievably expensive and came in very small quantities, which meant that vector batches all had to be compared. You might get twenty patients from a single batch, but equally, I know of someone who paid $2 million for a batch of vector from a third-party supplier and it produced one patient dose! We used to be on tenterhooks when the vector came back as to whether it would produce doses for one patient or for fifty. Sometimes it all went wrong and produced no doses at all. So we absolutely had to bring that in-house. We did so at Stevenage.

The next thing was the cell manufacturing. Some academic centers started building up their own cell manufacturing, as there was nowhere available to outsource. The University of Pennsylvania did the manufacturing for us to start with, and we got our vector from other academic suppliers in California.

Then we moved manufacturing to a big professional CMO, but we realized the technology transfer was not there. Academic processes do not really work when you move them outside that setting. A lot of cell and gene therapy companies have ended up having their own internal manufacturing capabilities, not because they wanted to build big infrastructure, but because they wanted the reliability.

At one point, the reliability of the external cell manufacture was running at 33%. That means the doctor was having to tell two in every three patients, after they had undergone an apheresis, that there was no product and therefore, no treatment. It is not like an aspirin where you just buy a different brand. For autologous cell therapies, if there are no cells, then there is no product.

> "...one of the issues with cell therapy is it is impossible to do toxicology studies in animals - even a chimpanzee doesn't have the same T cell receptor as a human."

We realized the emphasis had to be on reliability. We ended up bringing the vector in-house to expand it, make it cheaper, and work out exactly how to use it. Also, the cell manufacturing had to come back

"...the process is changing so much. It is becoming so much less manual and more automated. You can now just leave something to brew for 2 weeks, rather than having to go in and add reagents every day."

in-house, so we did not have patients being led through the process and then told that there were no cells, which was awful I am pleased to say that this year our manufacturing failure rate is extremely low. You have to get it to 100%.

The trends of the industry were driven by the inability to outsource. There are some transformative technologies coming through in almost every little aspect. But you cannot just change something and then say you have changed it – you have to measure it, and measuring a cell is very difficult.

External manufacturers have become much better and are much keener on doing things like vector production, because now there are more customers. The economics have changed to make it more realistic.

Looking forward twenty years, I would be rather surprised if Adaptimmune is manufacturing its own cells. This is because you will be able to employ contract manufacturers, particularly when the field becomes allogeneic. Being allogeneic means that you can actually afford product failures and still have a supply stream, because you can build up inventory.

Q What are your thoughts on likely successful strategies for the sector in addressing the challenge of sourcing, developing, and retaining a suitably skilled workforce?

JN: It is an industry issue that there are not enough people in certain areas, particularly manufacturing.

There were not enough people to make monoclonal antibodies 40 years ago, because there was nobody making them. People get more attracted to a sector as it grows. Eventually, you get hubs of expertise. Then, you get people who want to do that sort of thing coming to those areas, they become competitive, and the workforce expands from there as more and more people are trained. I would say it was harder right at the beginning because there were no managers or people doing the training.

We set it up independently and took our own route. We had to have a flexible workforce, because they needed to work shifts a lot of the time. Some of the processes take a long time and you couldn't have people going home in the middle of a process.

But the process is changing so much. It is becoming so much less manual and more automated. You can now just leave something to brew for 2 weeks, rather than having to go in and add reagents every day.

The fewer working parts, the better. We lost one cell lot years ago at a subcontractor because the cleaner pulled the plug out of the wall to clean behind the bioreactor one night. It was a wave bioreactor, which is a vulnerable type of machine; if it stops rocking, the cells

DOI: 10.18609/cgti.2022.016

are gone. You want things that are foolproof and do not require manual intervention. And of course, every manual intervention requires a batch record of what you have done, when, and why.

Q What are your thoughts on expanding patient access to cell and gene therapy products in future, and how will we get there?

JN: Access is about money because many countries cannot afford US prices. Even in the US, these are expensive drugs. It is going to come down to the universality of the treatment. First of all, you have to scale-up, and again, that means, in the short to medium term, improving cost of goods and efficiencies, and on the long term, allogeneic.

The decision we made to scale-up successfully in the autologous setting, has been to build an integrated company, where we can control all parts of the process and we can have a virtuous cycle of feedback between all parts of the business, to continually improve these processes. Essentially, when looking at allogeneic there are two ways to scale-up to a bank of cells from which you can make withdrawals. You can either get them from a donor, or you can develop them from stem cells. We looked at both options and chose the stem cell route. This was because the donor-derived cells do not produce huge batches, so you cannot really scale-up. There is also a limited number of donors who are eligible. The beauty of using stem cells is that you start off with a cell line that has been checked to the nth degree, which can then produce cells.

You have to take out a lot of the endogenous material – for example the existing TCR. Then, you put back in your genetic modifications. And at the end of each step, you can check whether you have actually got what you think you have got and be absolutely certain.

At the end of this process, you have a cell bank. There have been many problems and it takes a long time, but at Adaptimmune, we have put that time in. When we started, we internally gave it just a 5% chance of success. We thought if it worked, it would essentially take over cell therapy. Rather to our surprise, it has worked, despite taking a long time. We are now at the next stage: scaling it up.

Q Finally, what are your goals and priorities in your own work over the coming 1-2 years?

JN: At Adaptimmune, it will be a magic period when we can get our first autologous product approved, and we start our first allogeneic trial. We have overcome so many hurdles to get this far. We started with three post-docs at Oxford University, back in 2000, trying to exemplify a single patent, which turned out not to work anyway. So then we had to work out how to make it work, then how to engineer it, and eventually, how to treat people.

I have also really enjoyed looking at companies with great science or great ideas, but that need trans-Atlantic funding. In Europe, there is still piecemeal money going in. The scale

in the US is much greater, but not everybody can go to the US because you must have good enough product and a good enough management team. I have been spending my time trying to find management with a good enough set of products and science, but that have no idea about how to get onto the NASDAQ, for instance. I have essentially gone back to my original investment banking roots with the benefit of having accrued a bit of CEO experience in between.

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

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The Author is a Director of Adaptimmune Therapeutics PLC. The author owns stock and options in Adaptimmune. The author declares that they have no conflicts of interest.*

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

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited.*

Interview conducted: *Oct 13 2021;* ***Publication date:*** *Jan 24 2022.*

DOI: 10.18609/cgti.2022.016

CELL & GENE THERAPY INSIGHTS

BUSINESS INSIGHT: FEBRUARY 2022

COMMENTARY/OPINION

Regenerative medicine: new paradigms

"There is a lot to look forward to in the cell and gene therapy field. But there are a few key challenges that ARM and the sector are addressing."

Janet Lynch Lambert, CEO, Alliance for Regenertive Medicine (ARM)

2021 was an auspicious year for the regenerative medicine and advanced therapies sector. We are approaching a new frontier in the field, fueled by record-breaking investment and a host of anticipated near-term regulatory and clinical milestones. Against the backdrop of the global pandemic, we are challenging the scientific dogma to bring innovative therapies to a growing number of patients. As the global voice of the regenerative medicine and advanced therapy sector, the Alliance for Regenerative Medicine (ARM) tracks investment, clinical, and scientific trends that show the field is rapidly advancing. And a strong year in 2021 has set the stage for significant growth in 2022 and beyond.

Cell & Gene Therapy Insights 2022; 8(1), 53–58

DOI: 10.18609/cgti.2022.018

INVESTMENT LANDSCAPE

Investors are taking note of early successes in regenerative medicine. 2021 set another investment record, with $23.1 billion raised – a 16% increase over the previous record of $19.9 billion set in 2020 (Figure 1). Gene therapy developers raised the most with $10.6 billion, followed closely by cell-based immuno-oncology developers, who raised $10.1 billion. Cell therapy developers raised $2.0B and tissue engineered product developers raised $341M.

Companies active in gene editing have raised a growing proportion of investment – about one third of total investment in 2021. In particular, 45% of gene therapy financings this year were raised by companies active in gene editing – up from 38% three years ago. This trend likely reflects recent scientific and clinical advances in gene editing, including the announcement of the first in vivo CRISPR data in June of 2021, as well as the imminent advancement of technologies such as base editing into the clinic.

The record investment in 2021 was largely driven by venture capital, with $9.8 billion raised – a 73% increase over 2020. There were also a record number of 26 IPOs in 2021, eclipsing the previous record of 14 and raising a total of $4.8 billion.

However, the average value of an IPO dropped following the first quarter of the year, and the total amount raised in follow-on offerings decreased by 38% compared to 2020 – corresponding with lower public equity performance over the course of the year. Gene therapy – particularly AAV gene therapies – were the worst performers, dropping about 50% in value, compared to 4% for the NASDAQ biotech index and 22% for the XBI. But despite a rough 2021, RMAT – an index that reflects performance across all regenerative medicine technologies – still closed out the year at 177% of 2018 levels.

While activity in mergers and acquisitions (M&A) remained modest in 2021, we anticipate an upswing in 2022, including the closing of Novartis's $1.5 billion acquisition

FIGURE 1

Yearly investments in regenerative medicine (2017 – 2021)

DOI: 10.18609/cgti.2022.018

of ophthalmologic gene therapy developer Gyroscope Therapeutics.

COMMERCIAL LANDSCAPE

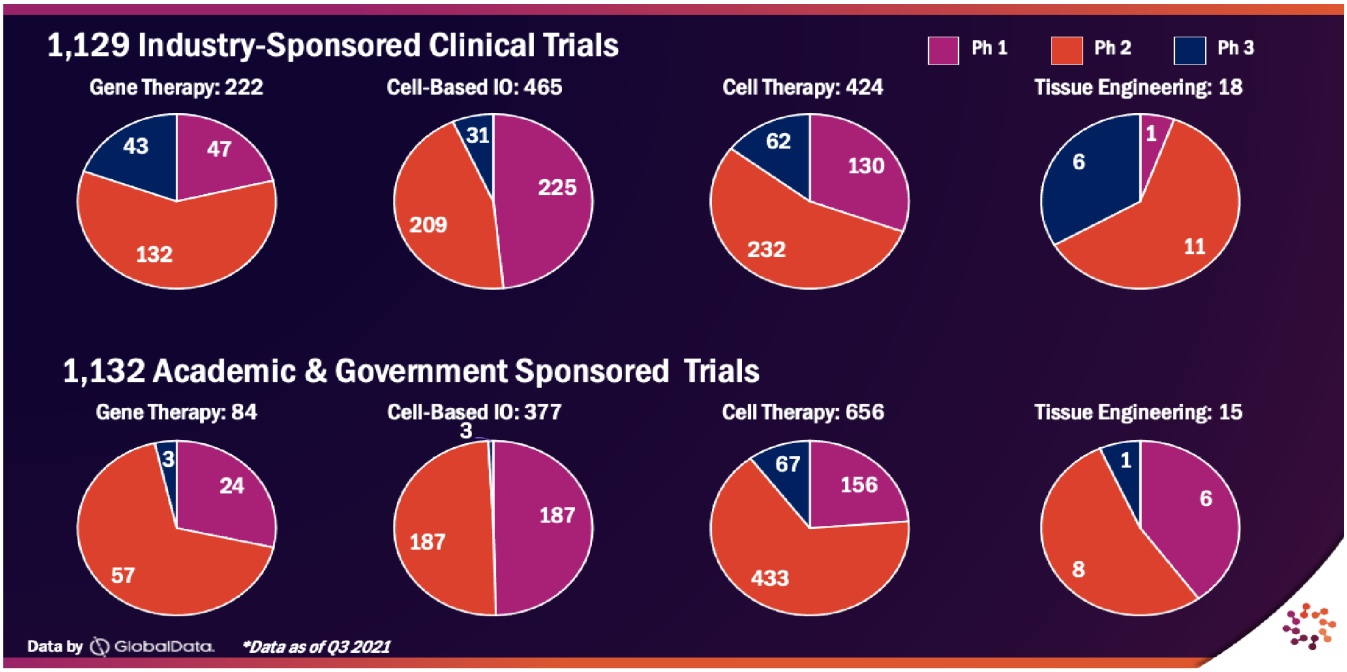
Turning to the commercial landscape, we can also see immense progress. Six new products were approved globally in 2021, making it the second-best year on record, following nine approvals in 2016. Those approvals include three new CAR-T therapies: bluebird bio and Bristol Myers Squibb's Abecma, the first BCMA-targeted CAR-T (which was also the first CAR-T for multiple myeloma), was approved in Europe and the US; Bristol Myers Squibb's Breyanzi for diffuse large B-cell lymphoma, approved in the US; and JW Therapeutics' Carteyva for large B-cell lymphoma, the second CAR-T therapy approved in China. Additionally, Mallinckrodt's tissue therapy Stratagraft for severe burns and Enzyvant's Rethymic for pediatric congenital athymia, a rare and historically fatal birth defect, were approved in the US. And bluebird bio's Skysona, for the serious inherited neurological disorder cerebral adrenoleukodystrophy, was approved in Europe.

It was a breakout year for the US FDA's Regenerative Medicine Advanced Therapy (RMAT) designation as well. This designation, which ARM advocated for on behalf of the sector, was established by the 21st Century Cures Act in 2016 and is intended to expedite patient access to innovative regenerative medicine products. Breyanzi became the very first product with RMAT designation to receive FDA approval, followed by Stratagraft and Rethymic.

As the field continues to mature, a growing number of developers are entering the space. There are 1,308 companies worldwide active in developing regenerative medicines and advanced therapies. The US, with 639 developers, and Asia-Pacific, with 410 developers, are driving growth in this arena – these regions have grown by 18% and 39% respectively compared to one year ago.

FIGURE 2

Industry, academic and government sponsered ongoing clinical trials as of 2021



CLINICAL & SCIENTIFIC LANDSCAPE

There are 1,129 industry-sponsored clinical trials ongoing globally (Figure 2). The largest proportion of those are in cell-based immuno-oncology (465) and cell therapy (424), followed by gene therapy (222) and tissue engineering (18). There are 403 in Phase 1, 584 in Phase 2, and 142 in Phase 3. Cell-based immuno-oncology continues to be one of the fastest-growing technology areas, with over half of Phase 1 trials (56%) utilizing this technology. ARM also for the first time this year reported on the 1,132 ongoing government and academic-sponsored clinical trials in the sector. As a whole, these trials tend to be earlier stage than industry trials, with a larger proportion of cell therapy trials and a much smaller proportion of gene therapy trials.

Cancer remains the number one target for the cell and gene therapy sector, with 1,354 industry, academic, and government sponsored trials ongoing. And while early therapies to market focused on treating cancer after three or more previous lines of treatments failed, data this year from Gilead and Bristol Myers Squibb suggest that these therapies can perform favorably compared to the standard of care in earlier-line treatments. There's also an uptick in interest from companies like Sana Biotechnology that are looking to modify CAR-T therapies *in vivo.*

Regenerative medicine companies have historically focused on monogenic rare diseases. But more complex, polygenic diseases are increasingly becoming targets. This year, we saw a promising data readout from Vertex in which a stem cell therapy appears to have cured a patient's type 1 diabetes. And experimental therapies using pluripotent stem cells are being tested to treat diseases like Parkinson's. Looking at upcoming clinical data readouts, while most Phase 3 readouts are focusing on rare diseases and liquid tumors, we see a greater proportion of complex, more prevalent diseases in Phase 1 and 2.

Vertex's diabetes readout was also significant because it challenged conventional wisdom in the cell and gene therapy space: that damage that has already been done cannot be reversed. Additionally, a clinical trial in AADC deficiency found that a gene therapy administered to an eight-year-old girl with the disease was able to undo devastating neurological effects. The patient can now walk unassisted, and is beginning to use a speech-generating device. Together, these two readouts suggest that there may be a wider window than previously thought during which a disease can be altered or even reversed.

Noted previously, gene editing is continuing to gain prominence, with the first data from an *in vivo* CRISPR trial reported last summer and new base editing trials likely to enter the clinic later this year. But new strategies for genetic engineering that go beyond changing the genetic code itself are emerging: developers are looking into changing methylation, "squeezing genes," and epigenetic editing to produce therapeutic benefits.

LOOKING AHEAD TO 2022 & BEYOND

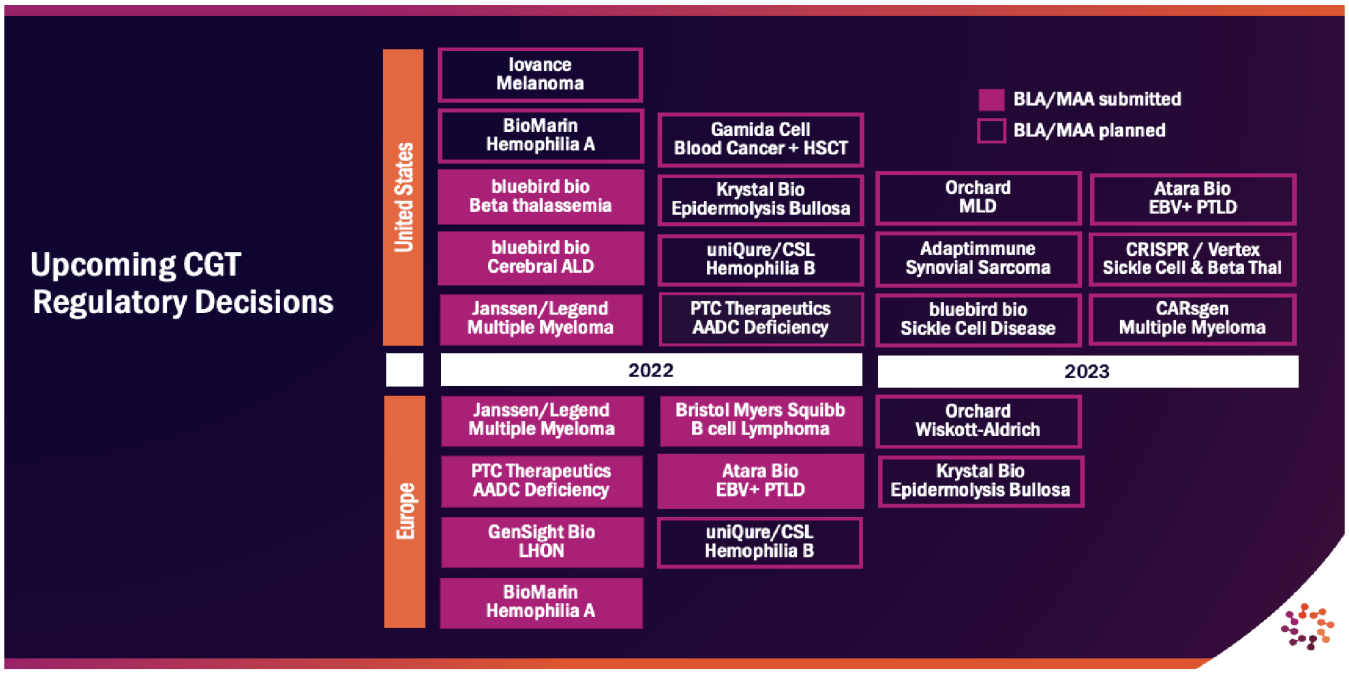
2022 is expected to be a banner year for gene therapy for rare diseases. Five new such gene therapies are up for regulatory decisions, including gene therapies for hemophilia A and B (BioMarin and uniQure/CSL Behring, respectively), AADC deficiency (PTC Therapeutics), Leber hereditary optic neuropathy (GenSight Biologics), and epidermolysis bullosa (Krystal Bio) (Figure 3).

Data readouts are expected in Phase 3 trials targeting hemophilia, Duchenne muscular dystrophy, and others, as well as additional data from Vertex's Phase 1 diabetes trial. Gene editing will continue its march to the clinic, with trials of new base editing technologies from Beam Therapeutics in sickle cell disease and Verve Therapeutics in heterozygous familial hypercholesterolemia. ViaCyte and CRISPR's gene editing therapy for diabetes is also poised to enter the clinic.

DOI: 10.18609/cgti.2022.018

FIGURE 3

Upcoming CGT regulatory decisions in the US and Europe



Mergers and acquisitions are expected to pick up, and positive data readouts could cause the early-stage investment picture and equity performance to align in a positive direction, especially if inflation concerns begin to subside.

But as the sector continues to challenge the boundaries of what was thought possible, what can be expected in the coming years?

Looking at the clinical pipeline and beyond, there will be a gradual evolution from rare monogenic diseases and liquid tumors to more complex diseases and solid-tumor cancers. Importantly, the first gene therapy for a prevalent disease could be just a few years out. The often-quoted prediction from the FDA in 2019 that it expected to approve 10–20 cell and gene therapy products annually by 2025 looks doable, although perhaps it will be at the lower end of that projection.

OVERCOMING KEY CHALLENGES

There is a lot to look forward to in the cell and gene therapy field. But there are a few key challenges that ARM and the sector are addressing.

While safety concerns – particularly around AAVs – weighed down the sector last year, there is reason for optimism. Thousands of patients have been treated safely with AAV-driven therapies. The FDA has signaled that it doesn't plan significant regulatory changes, particularly around dosage caps, and will work collaboratively with industry on important measurement techniques. And advances in regenerative medicine not only means improving AAVs, but also includes exciting research into other delivery vehicles and technologies for genetic medicines.

CMC remains a key challenge for the sector, but it is one we are addressing head-on. Expected Congressional legislation sponsored by US Representatives Diana DeGette (D-CO) and Fred Upton (R-MI) – should be helpful in bringing about more clarity on CMC requirements, as well as the PDUFA VII agreement. ARM continues to work directly with the FDA for improved clarity. Additionally, in 2021, we released Project A-Gene, a best practices guide to using quality by design (QbD) manufacturing standards for gene therapy, which is now becoming widely used as a workforce development tool.

The final challenge is policy and reimbursement. For Europe in particular, 2022 will be a big moment for the region to address declining competitiveness. Legislation emanating from the EU Pharma Strategy will represent the first major review of the pharma landscape in Europe in 20 years, and ARM is working to make sure ATMPs can thrive. In the US, we must remove barriers to innovative payment models, ensure that policymakers understand that this sector is fundamentally different from traditional pharmaceuticals, and reorient our healthcare system around value instead of cost.

We are on the precipice of the next generation of regenerative medicine and advanced therapies, which will leverage cutting-edge technologies to benefit millions of patients worldwide. The biggest threat to the promise of regenerative medicine isn't the science – it is 20th century regulatory and reimbursement challenges that can delay this 21st century medicine. ARM looks forward to working with our members and with stakeholders from across the sector to ensure the science advances and patients can access these innovative therapies.

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AUTHORSHIP & CONFLICT OF INTEREST

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

Acknowledgements: *None.*

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

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

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *Jan 25 2022*; ***Revised manuscript received:*** *Jan 27 2022*; ***Publication date:*** *Feb 2 2022.*

DOI: 10.18609/cgti.2022.018