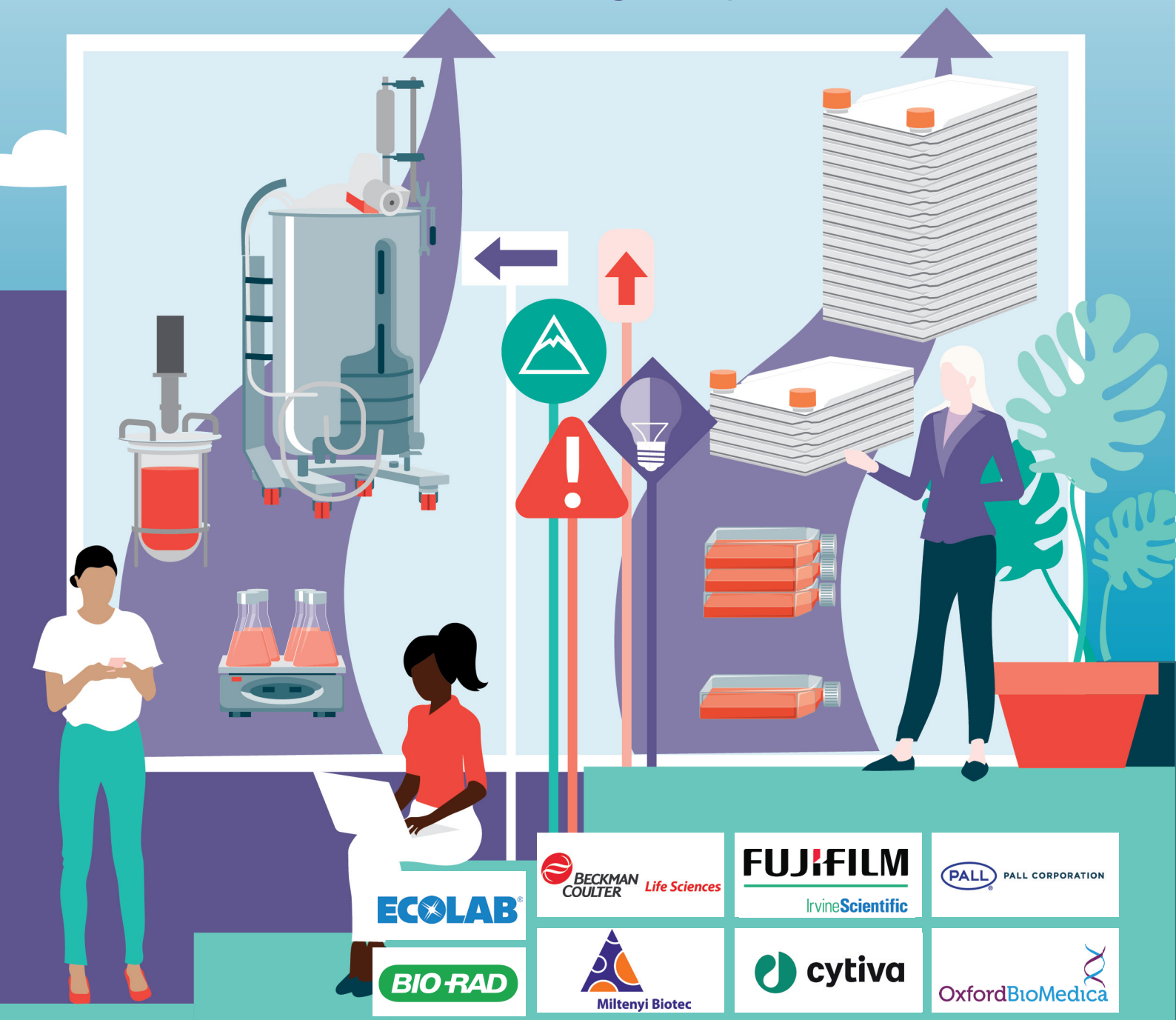
SEPTEMBER 2022 Volume 8, Issue 8

CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON:
Cell and gene therapy manufacturing scale-up/scale-out

Guest Editor: Nolan Sutherland, Ring Therapeutics



CELL & GENE THERAPY INSIGHTS Volume 8, Issue 8

CONTENTS

SPOTLIGHT: CELL & GENE THERAPY MANUFACTURING SCALE-UP/SCALE-OUT
LATEST ARTICLES
ANALYTIC CHANNEL EDITION

Spotlight

Cell & Gene Therapy Manufacturing Scale-Up/Scale-Out

Latest articles

CELL & GENE THERAPY INSIGHTS

Volume 8, Issue 8

Analytic Channel

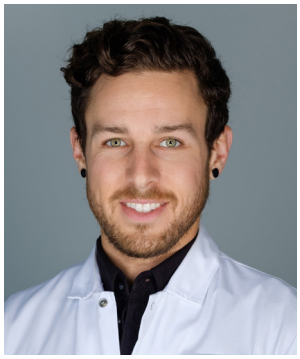
CELL & GENE THERAPY INSIGHTS

CELL & GENE THERAPY MANUFACTURING
SCALE-UP/SCALE-OUT

SPOTLIGHT

De-risking late-stage activities by retaining your early-stage expertise

Nolan Sutherland
Senior Scientist, Process Development, Ring Therapeutics, Inc.



"...as innovators in science and technology we should apply the same 'thinking outside the box' strategy to managing our expertise"

EDITORIAL

Cell & Gene Therapy Insights 2022; 8(8), 921–923

DOI: 10.18609/cgti.2022.137

The topics of scaling-up and scaling-out have never been so sexy in the biotechnology industry. The world of cell and gene therapy GMP manufacturing is populated with cutting-edge processes, incorporating new and exciting technologies at each step. However, the large-scale production and purification of cell and gene therapy (CGT) products can include highly complicated operations, process intermediates that are sensitive to degradation, and labile final products. Allow the cells sit too long? Batch gone. Allow a transfection reagent to mix for too long? Batch gone. Product degrading over the course of final vial inspections? Sorry. It's the manufacturing world we currently live in where we must rely on process control and knowledge to harness the power of biology. For Process Development teams, the exercise of scaling a cell or vector process from the bench to GMP manufacturing is challenging, exciting and very rewarding if successful. Biological systems are chaos at their core, and we are pushing the boundaries of what is operable in a manufacturing suite.

These processes have now made their way to their proving ground, with commercialization well underway within many late-stage companies. With this, we've started to see where "the bar" is being set in terms of approvals. This has also afforded us all the opportunity to witness a Refusal-to-file notice issued in the CGT space due to a lack of detail in a Chemistry, Manufacturing and Control (CMC) package [1], a decision by the FDA that has historically been based on product efficacy and/or quality. The Food and Drug Administration (FDA) appreciates the complexity of what we're doing and is ensuring each filing encompasses a deep process understanding.

We've also seen how these outcomes impact companies on a whole, where running towards a product approval based on clinical results leaves very little room for error in manufacturing results. Setbacks that ensue from CMC failures hurt company morale, external trust, and unfortunately stock price. So what can the industry do to de-risk these late-stage challenges and ensure stronger filings? My answer isn't novel but needs to be reiterated, retain expertise.

The characterization that CGT processes are innately complicated and a challenge to execute would make the uninitiated believe that batch success is chance. Chance that the cells grow correctly, the transfection works or the transducing units you calculated and added are correct and active. Some is due to the inherent variability of operating biology in situ, but if you have incorporated the right tools to control batch production and monitor, measure, analyze and release your product then the outcome of batch success is calculable. What this calculus relies on, however, is always having the expertise to know exactly what those tools are, when they should be used, why they are being used, and how they impact the product.

During the recombinant enzyme and monoclonal antibody boom, once a process was 'late-stage' it could be operated by anyone with a Batch Record. This is not the reality of CGT products. Batch Records capture 'What to do' and 'When to do it', but when a deviation arises in these processes that requires diligent thought on how the next steps can impact the product, you need an expert in the room. What I've seen is the talent that develops these processes doesn't always stick around for commercialization, leaving huge gaps in process understanding. It's no surprise that the ones who met the challenge of developing these processes don't find the same satisfaction in characterizing, validating, and monitoring them, but they are essential when addressing regulators on process-based questions and ensuring batch success in later stages of development.

Retaining expertise de-risks late-stage CGT activities greatly and can be achieved through baking it into your company's commercialization strategy. The notion is often exclusively tied to people, but retaining expertise extends to systems and culture, as well. Promote knowledge flow, cross-functional training, internal capturing and presentation of important data and results. Invest in robust digital

DOI: 10.18609/cgti.2022.137

systems for R&D that add efficiency to accessing technical information. These can greatly enhance understanding of key concepts and historical knowledge across the board. And of course, do your best to retain your subject matter experts (SMEs). This is not advocating for keeping people around for the sake of it, it's promoting the idea that we should recognize who our SMEs are and craft ways to keep them engaged throughout a processes' life cycle. There seems to be a whole industry today devoted to improving employee recognition and job satisfaction from which to pull, and decades of previous development cycles from other sectors of biotech from which to learn. But as innovators in science and technology we should apply the same 'thinking outside the box' strategy to managing our expertise as developing it.

BIOGRAPHY

NOLAN SUTHERLAND graduated from the University of Massachusetts, Amherst with a BSc in Biochemistry and Molecular Biology where his research areas of focus were on molecular cloning and RNAi. Over the past 10 years he has worked in both Upstream and Down-stream Process Development groups, getting his start in the area of enzyme replacement therapies before entering the gene therapy field at bluebird bio. Since that time, the majority of Nolan's work has been focused on developing suspension-based lentiviral vector production systems for commercial manufacturing. His areas of expertise include process establishment and improvement at the miniature and bench-scales, emerging technology evaluations, transient transfection optimization, nanoparticle analysis and characterization, as well as scale-up and technology transfer. With a new role in viral vector R&D, he aims to support the development of novel vectors for *in vivo* use.

REFERENCE

1. Bristol Myers Squibb and bluebird bio Provide Regulatory Update on Idecabtagene Vicleucel (ide-cel, bb2121) for the Treatment of Patients with Multiple Myeloma. Bristol Myers Squibb 2020 (05/13/2020). (Accessed Aug 2022).

AFFILIATION

Nolan Sutherland
Senior Scientist, Process Development,
Ring Therapeutics, Inc.

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 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; externally peer reviewed.*

Revised manuscript received: *Aug 22 2022*; ***Publication date:*** *Aug 31 2022.*

CELL & GENE THERAPY INSIGHTS

CELL & GENE THERAPY MANUFACTURING SCALE-UP/SCALE-OUT

SPOTLIGHT

EXPERT INSIGHT

Stabilizing DNA–PEI complexes improves scalability of suspension lentiviral viral vector and AAV processes

Brynn Olden, Hannah Seo, Robert Barnes, Kimberlee Sing, Catherine Ludolph, & John Moscariello

Transient transfection is a challenging and time-sensitive upstream unit operation to scale for suspension HEK293 viral vector production. For many transfection reagents, including polyethyleneimine, careful timing of mixing and hold steps is essential to process performance but challenging to maintain at relevant manufacturing scales. We first characterized the process parameters that influence polyethyleneimine transfection reagent performance and then implemented a stabilizing strategy to preserve the activity of transfection complexes when scaling. Generating a transfection complex with an average hydrodynamic diameter between 500 and 700 nm was most predictive of transient transfection productivity, measured by viral vector titer, but is very sensitive to transfection complex concentration and hold time. The addition of human serum albumin as a stabilizing agent maintained the optimal transfection complex size for extended periods of time and improved the process control for both lentiviral vector and adeno-associated viral vector suspension processes at multiple production scales.

Cell & Gene Therapy Insights 2022; 8(8), 985–992

DOI: 10.18609/cgti.2022.146

INTRODUCTION

Viral vectors, including lentiviral vectors (LVV) and adeno-associated viral vectors (AAV), continue to be instrumental for both cell and gene therapies [1,2]. A major challenge involved in developing an effective HEK293 suspension-based cell culture process for viral vector production is maintaining

transient transfection productivity when scaling up. The transfection unit operation is a crucial step in developing a suspension process and has a high impact on harvest titers and resulting process yields [3]. Cationic polymer-mediated (e.g., polyethyleneimine, [PEI]) transient transfection is the industry standard method for introducing plasmid DNA containing viral vector genes into host cells to induce vector production and is a proven effective technique for suspension production processes [4–6].

For suspension process scale-up, it is desirable to hold PEI and DNA concentrations and complex hold times constant. Concentration can be held constant by linearly scaling up the volume of the transfection complex with the bioreactor size. However, maintaining a constant hold time is more challenging to scale due to differences in time required to mix solutions at different scales and the speed at which large volumes can be transferred. This presents a challenge at larger volumes as the stability of PEI and DNA transfection complexes formulated in cell culture media is often on the order of minutes and these large volume mixtures cannot be physically transferred into the bioreactor fast enough to maintain consistent, high titer performance. This creates a high risk for process variability and loss in volumetric productivity upon scale-up of suspension viral vector processes or sacrificing harvest titers for improved process robustness.

In recent years, many groups have found innovative ways to gain additional control over transient transfection processes, mostly focused on optimizing transfection complex size and improving transfection complex stability over long time periods. These approaches have included developing new transfection reagents with vendor-reported extended hold times, modulating the transfection complex surface charge and particle association via pH and salt concentration shifts, as well as shielding particles with an additional reagent to form stable ternary complexes [7–10].

Here we share our work investigating the optimal range of PEI and DNA complex size for LVV and AAV production in a suspension HEK293 cell culture system, as well as demonstrating the effectiveness of human serum albumin (HSA) to stabilize transfection complexes and improve scalability of the transient transfection unit operation with commercially available cGMP-compliant reagents.

METHODOLOGY

PEI–DNA complex sizing

Working solutions of PEIpro (Polyplus) and plasmid DNA (Aldevron) were prepared by diluting stock solutions into chemically defined serum-free HEK 293 cell culture media to the desired concentration. PEI–DNA complexes were analyzed by dynamic light scattering (DLS) using a Malvern Zetasizer instrument. All materials were allowed to equilibrate to room temperature (approx. 20°C) prior to use. All samples were prepared in a 10 × 10 × 45 mm square bottom polystyrene cuvette (Sarstedt REF# 67.754). For kinetic studies, 15 second size measurements were taken for the first 5–10 min, after which 60 second size measurements were taken every 3–5 min until study completion. For conditions where HSA (Octapharma) was added, a concentrated solution of HSA was added directly into the PEI–DNA complex measurement cuvette at the optimal time in between measurement intervals.

HEK293 transient transfection

Suspension HEK293 cells were cultured to optimized cell density in shake flasks or single-use bioreactors (1 L Eppendorf DASGIP or 10 L Eppendorf BioFlo system). Working solutions of PEIpro and plasmid DNA were prepared by diluting stock solutions into cell culture media to the desired concentration.

For LVV production, a third generation LVV 4-plasmid system was used where the 4 plasmids encoded VSV-G envelope, gag-pol, rev, and gene of interest (GOI). For AAV

DOI: 10.18609/cgti.2022.146

production, a 3-plasmid system was used where the three plasmids encoded helper, RepCap, and GOI. Optimized plasmid ratios were used for both LVV and AAV production.

The working solution of PEI was added to the working solution of DNA at a 1:1 volumetric ratio and mixed by pipetting or swirling. This transfection complex mix was statically held for the specified hold time before being added to the HEK293 cell culture. For conditions where HSA (Octapharma) was added, a concentrated solution of HSA was added directly into the PEI–DNA complex at the optimal time to achieve a final concentration of 1 mg/mL HSA and then statically held for the specified hold time before being added to the HEK293 cell culture. For shake flask studies, transfection complex was added to the culture via pipetting. For bioreactor studies, transfection complex was added to the culture via gravity draining through an overlay tubing line.

For LVV production, samples were collected at the optimized culture duration post-transfection, clarified by centrifugation, and supernatant was stored at -80°C until testing.

For AAV production, at the optimized culture duration post-transfection, samples were treated with a lysis buffer and nuclease enzyme, clarified either by centrifugation or syringe filtration, and stored at -80°C until testing.

LVV titer

Jurkat cells were transduced with a CAR-encoding LVV-containing sample and measured for CAR expression using flow cytometry. Titer of the sample was extrapolated from %CAR+ Jurkat cells and quantified as transducing units/mL (TU/mL).

AAV titer

Quantitative polymerase chain reaction (qPCR) of lysed adeno-associated viral vector samples used primer probes targeting a promoter sequence in the vector genome to amplify and quantify the vector genome. Titer results are reported as vector genomes/mL (vg/mL).

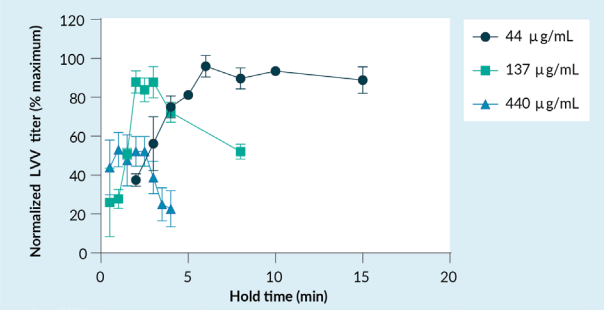
RESULTS

PEI–DNA complex size impacts transient transfection harvest titers of LVV and AAV

During process development for suspension HEK293 cell culture processes for LVV and AAV production, it was observed that vector titer was sensitive to PEI–DNA transfection complex hold time prior to addition to the HEK293 cell culture production vessel. Knowing this would become a challenge to maintain as the process scaled to larger volumes, the concentration of the transfection complexes was increased with the goal of reducing the volume of transfection complex required to be added to the production bioreactor, reducing the volumetric transfer time. However, the hold time-sensitivity increased as the concentration of the transfection mixture increased, and the precision required to meet a short process step duration was going to compromise process robustness in a manufacturing environment (Figure 1).

FIGURE 1

Transfection complex hold time and concentration impact on LVV harvest titer. Error bars represent standard deviation based on N=3 replicates.



PEI–DNA complex size is controlled by complex hold time and complex concentration

Based on the mechanism of action of PEI complexes to facilitate charge-mediated endocytosis of its cargo, it was hypothesized that the sensitivity to hold time was due to the PEI–DNA complex size and ability for the transfection complexes to efficiently be endocytosed by the HEK293 cells [11]. To test this hypothesis, PEI–DNA complex size was measured over time at multiple concentrations via dynamic light scattering (DLS) (Figure 2A). This study confirmed that PEI–DNA complex size and growth kinetics were dependent and positively correlated to both complex concentration and hold time, where complex size increases over time and at a faster rate at a higher concentration. When transfection complexes of different concentrations and hold times were used to produce AAV, titer results held a similar trend to what was seen previously for LVV (Figure 1), where there was a different optimal hold time for different transfection complex concentrations (Figure 2B). Plotting AAV titer versus estimated complex size (calculated using complex hold time and concentration data shown in Figure 2A), it was clear that the maximum titer was achieved with a PEI–DNA complex size in the 500–700 nm range across all complex concentrations tested (Figure 2C).

Human serum albumin stabilizes PEI–DNA complexes at optimal complex size for extended time periods

Prior experience developing adherent LVV processes where fetal bovine serum (FBS) containing media was often used to 'quench' calcium phosphate transfection complex growth inspired a similar solution for stabilizing PEI–DNA transfection complexes in a serum-free chemically defined media used for suspension cell culture LVV production. It was hypothesized that introducing human serum albumin (HSA) could have a similar stabilizing effect for PEI–DNA complexes as observed from FBS in calcium phosphate transfections. To test this hypothesis, PEI–DNA complexes for LVV production were formed and measured using dynamic light scattering during which concentrated HSA was added to the transfection complex mixture at the previously defined optimal hold time based on the prior viral vector production experiments performed using transfection complexes without HSA. For transfection complexes with optimal hold times greater than 2 min, HSA was able to stabilize the transfection complex size for 4 h, which was the longest time duration measured on DLS (Figure 3). For highly concentrated transfection complexes, the timing of the HSA was likely too late to prevent transfection complex aggregation as the complex size had already grown outside of the optimal range (> 1000 nm).

Stabilized PEI–DNA complexes provide improved process control & scalability of transient transfection for LVV and AAV manufacturing

Human serum albumin stabilized PEI–DNA transfection complexes were then used in the lab-scale production of both LVV and AAV. At the shake flask scale, HSA stabilized transfection complexes could be held at room temperature for up to 2.5 h without any appreciable negative impact on viral vector titer (Figure 4). This was observed in both LVV and AAV processes using different optimal cell culture media, cell densities, and transfection complex concentrations, demonstrating the versatility of this process control strategy.

Finally, scalability was demonstrated by producing LVV at the 1 L and 10 L bioreactor scales where harvest titers were maintained for transfection complexes held for 30 min (Figure 5A) and across multiple independent runs at each scale as compared to representative shake flask experiments (Figure 5B).

DOI: 10.18609/cgti.2022.146

FIGURE 2

Transfection complex size screening on AAV production. A. Complex size (z-average) over time at different concentrations measured by DLS. B. Transfection complex size and hold time impact on AAV genome titer. C. AAV genomes harvest titer for transfection complexes of varying estimated sizes.

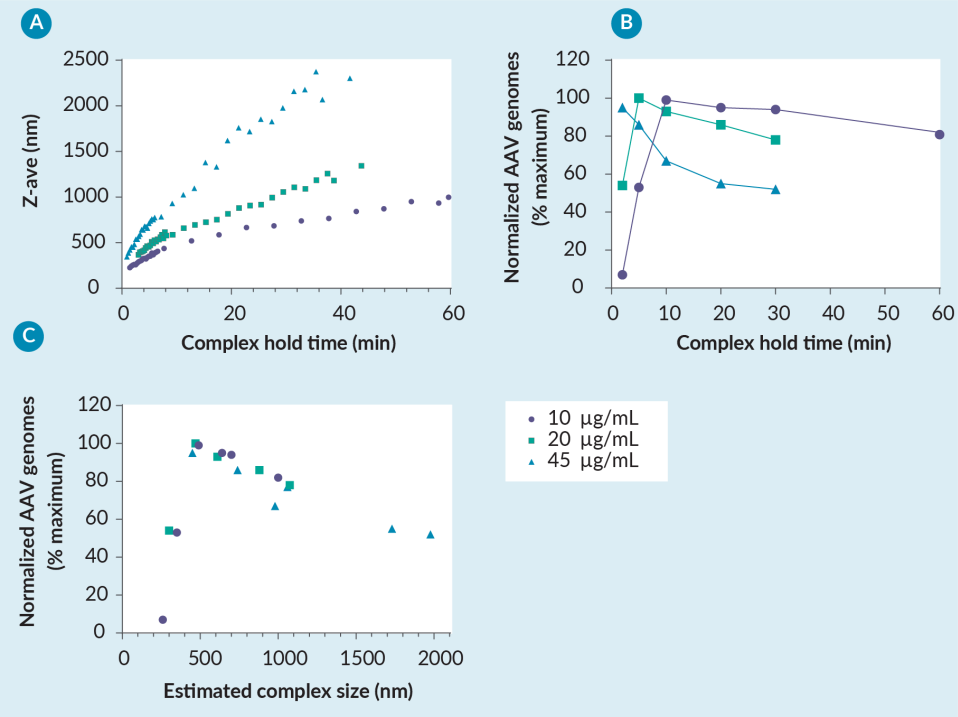
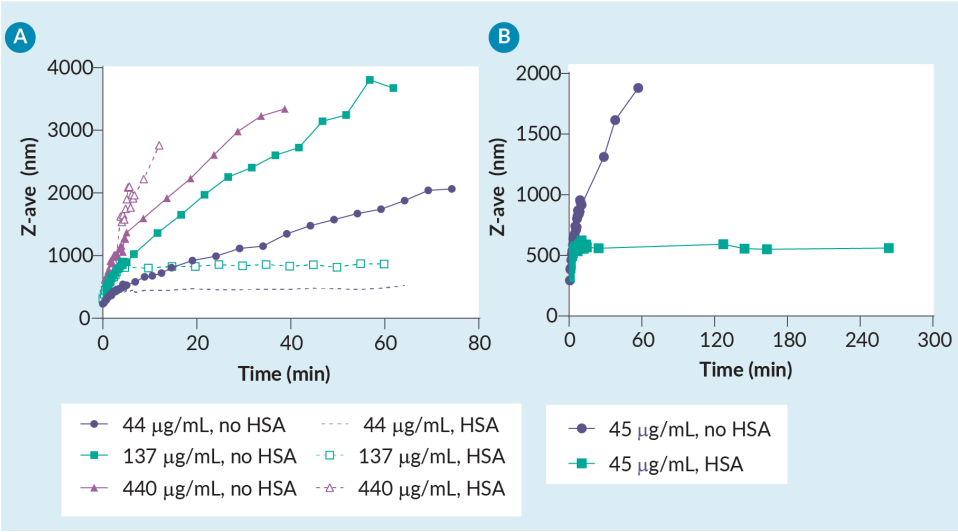


FIGURE 3

Transfection complex size stabilization with human serum albumin. A. Transfection complex size over time at different complex concentrations, with and without HSA. B. Transfection complex size over time, with and without HSA.



CONCLUSION

Maintaining productivity of suspension HEK293 cell culture to produce both LVV and AAV is dependent on consistent control of the transfection unit operation during process scale-up. The time-sensitive nature of PEI–DNA transfection complex efficiency

FIGURE 4

Transfection complex stability impact to viral vector harvest titers. A. LVV titer at various transfection complex hold times, with and without HSA. Error bars represent standard deviation based on N=3 replicates. B. AAV genomes titer at various transfection complex hold times, with and without HSA. Error bars represent standard deviation based on replicates.

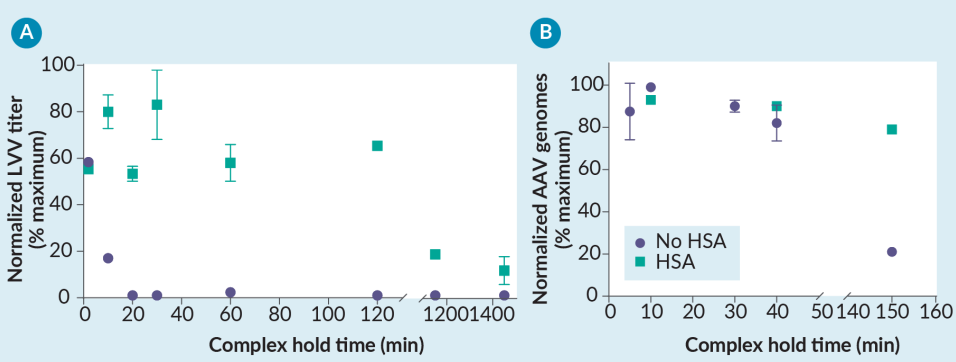
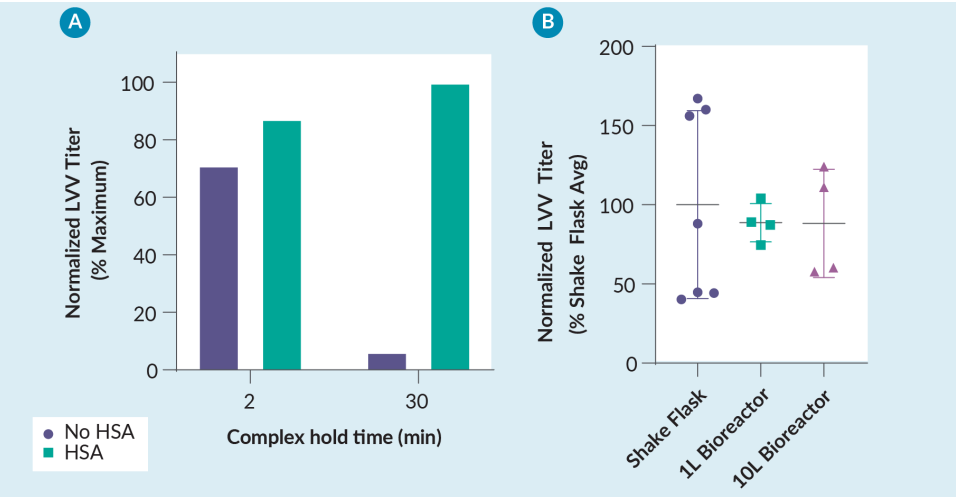


FIGURE 5

Scalability of HSA stabilized transfection complexes. A. LVV harvest titer in 1L stirred tank bioreactor (N=1). B. LVV harvest titer at shake flask, 1L bioreactor, and 10L bioreactor scale using HSA stabilized transfection complexes, normalized to average harvest titer from shake flask replicates.



poses a challenge to scaling and reduction in harvest titers can result if narrow hold time durations are not met. Both transfection complex concentration during mixing and hold time before adding the transfection complexes to the HEK293 cell culture are critical process parameters that contribute to transfection complex size and formation rate. An optimal transfection complex size of 500–700 nm resulted in the highest harvest titers in both LVV and AAV production processes despite differences in other process parameters across these two manufacturing processes (cell culture media, cell density, and transfection complex concentrations). Human serum albumin successfully stabilized transfection complexes within this ideal size range for up to four hours, significantly improving the manufacturability and consistency of the transfection unit operation in viral vector production. This ternary PEI–DNA-HSA transfection complex is a straightforward, commercially available, and cGMP-compliant solution for improving transfection unit operation consistency when scaling suspension HEK293 cell culture

DOI: 10.18609/cgti.2022.146

transient transfection viral vector processes for applications in both cell and gene therapy. Developing a similar approach using recombinant human albumin would be a potential alternative for animal-origin free processes. In the current state, this approach is especially appropriate for LVV and AAV products used to manufacture cell therapy products where human serum-derived reagents are commonly used in manufacturing.

REFERENCES

1. Bulcha JT, Wang Y, Ma H, Tai PWL, Gao G. Viral vector platforms within the gene therapy landscape. *Signal Transduct. Target Ther.* 2021; 6(1) 53.
2. Labbé RP, Vessillier S, Rafiq QA. Lentiviral vectors for t cell engineering: Clinical applications, bioprocessing and future perspectives. *Viruses* 2021; 13(8), 1–22.
3. Hildinger M, Baldi L, Stettler M, Wurm FM. High-titer, serum-free production of adeno-associated virus vectors by polyethyleneimine-mediated plasmid transfection in mammalian suspension cells. *Biotechnol. Lett.* 2007; 29(11) ,1713–1721.
4. Chahal PS, Schulze E, Tran R, Montes J, Kamen AA. Production of adeno-associated virus (AAV) serotypes by transient transfection of HEK293 cell suspension cultures for gene delivery. *J. Virol. Methods* 2014; 196, 163–173.
5. Segura MM, Garnier A, Durocher Y, Coelho H, Kamen A. Production of lentiviral vectors by large-scale transient transfection of suspension cultures and affinity chromatography purification. *Biotechnol. Bioeng.* 2007; 98(4), 789–799.
6. Nyamay'Antu A, Kedinger V, Erbacher P. Addressing scaling-up limitations: optimized PEI-mediated production of clinical grade viral vectors. *Cell Gene Ther. Insights* 2018; 4, 71–79.
7. Ito T, Iida-Tanaka N, Koyama Y. Efficient in vivo gene transfection by stable DNA/PEI complexes coated by hyaluronic acid. *J. Drug Target* 2008; 16(4), 276–281.
8. Hu Y, Zhu Y, Sutherland ND, Wilson DR *et al.* Size-Controlled and Shelf-Stable DNA Particles for Production of Lentiviral Vectors. *Nano. Lett.* 2021; 21(13), 5697–5705.
9. Nakamura Y, Sato H, Nobori T *et al.* Modification of ligands for serum albumin on polyethyleneimine to stabilize polyplexes in gene delivery. *J. Biomater. Sci. Polym. Ed.* 2017; 28, 1382–1393.
10. Rhaese S, Briesen H Von, Rubsamen-Waigmann H, Kreuter, Langer K. Human serum albumin – polyethylenimine nanoparticles for gene delivery. *J. Control Release* 2003; 92(1), 199–208.
11. Bus T, Traeger A, Schubert US. The great escape: How cationic polyplexes overcome the endosomal barrier. *J. Mater. Chem. B.* 2018; 6(43), 6904–6918.

AFFILIATIONS

Brynn Olden
Cell Therapy Development, Bristol Myers Squibb

Hannah Seo
Cell Therapy Development, Bristol Myers Squibb

Robert Barnes
Cell Therapy Development, Bristol Myers Squibb

Kimberlee Sing
Cell Therapy Development, Bristol Myers Squibb

Catherine Ludolph
Cell Therapy Development, Bristol Myers Squibb

John Moscariello
Cell Therapy Development, Bristol Myers Squibb

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: *The Authors would like to acknowledge Beth Larimore, Willard Foss, and Julie Shi for their intellectual contribution to this work.*

Disclosure and potential conflicts of interest: *The authors disclose that Bristol Myers Squibb owns patents relevant to this content. Robert Barnes has stocks in Bristol Myers Squbbs as part of an employee compensation package. 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: *Jul 11 2022*; ***Revised manuscript received:*** *Aug 29 2022*; ***Publication date:*** *Sep 7 2022.*

CELL & GENE THERAPY INSIGHTS

CELL & GENE THERAPY MANUFACTURING SCALE-UP/SCALE-OUT

SPOTLIGHT

INTERVIEW

De-risk & accelerate the drug development process for gene therapy

David McCall, Editor, *Cell & Gene Therapy Insights*, talks to **Kim Watanabe,** General Manager & Site Head, Patheon Translational Services, ThermoFisher Scientific

KIM WATANABE, PhD is the General Manager, Site Head for Patheon Translational Services. As part of the pharma services contract development and manufacturing organizational arm of Thermo Fisher Scientific, she leads the newly launched business that provides discovery stage, clinically bound clients with molecular biology, viral vector, and cell therapy services for *in vivo* gene therapy and *ex vivo* modified cell-based therapy research applications. Since joining Thermo Fisher Scientific in 2016, she has held multiple roles with increasing responsibility in product management and business integration and development. Prior to her current tenure, she was a Senior. Global Product Manager at Irvine Scientific (now Fujifilm Irvine Scientific), where she focused on cell culture products to support cell therapy applications. She was a stem cell research scientist at ScienCell Research Laboratories. Dr. Watanabe was a postdoctoral scholar at the University of California, San Diego, and received her doctoral degree in microbiology from the University of Virginia, and her BSc in cell and structural biology from the University of Illinois at Urbana-Champaign.

Cell & Gene Therapy Insights 2022; 8(8), 1129–1133

DOI: 10.18609/cgti.2022.166

Q What are some key high-level trends in the gene therapy sector that are impacting biotech decision-making around outsourcing?

KW: From my perspective, there are three main themes: Speed, Simplicity, and Resilience.

There are a significant number of advanced therapy sponsors who are small- to mid-sized startup biotech companies. Unlike big pharma companies, they are challenged by limited capital, tight timelines, and an incomplete understanding of what it means to establish a functioning, good manufacturing practice (cGMP)-compliant operation.

We live in an impatient world now more than ever and with the increasing degree of competition in the market for funding, investors in particular do not have a lot of patience. They want to see an almost immediate return on their investments. This means there is not much time to build a facility, especially when facing construction material shortages from the impact of the COVID-19 pandemic. Most importantly, it takes time to outfit the facility with the right group of talent. As a result, the need to outsource to experienced contract development manufacturing organizations (CDMOs) with the right set of expertise will continue to grow for small companies seeking to gain that maximal speed to commercialization, and ultimately, a faster path to their payday.

Another trend I have seen is the greater demand for supply chain simplicity and resilience to overcome current raw material bottlenecks. There are inherent workflow complexities in manufacturing cell and gene therapy products, as we are dealing with different vendors to procure hundreds of raw materials, many of which are unfortunately single source. There is a consistent need to find areas to improve the supply chain and to build a more streamlined process.

We, as a sector, learned a lot from our recent experience of the COVID-19 pandemic, where the macro economy came to a grinding halt. Drug developers are now putting more weight on logistics and raw material inventory management as part of their decision-making process. Consequently, we will see a greater emphasis on partnering with CDMOs that can offer a true end-to-end solution for support, as a one-stop-shop.

Q When should small biotechs engage with CDMOs?

KW: The earlier the engagement with an experienced CDMO, the better. There is a common misconception that small biotechs and drug developers cannot afford to engage with CDMOs at early stages of development due to enormous costs. There is an inclination from drug developers to wait until their lead candidate molecule is fully defined, and they have a good mapping of their chemistry, manufacturing, and controls (CMC) strategy.

Unrealistic expectations from both the drug developers and an inexperienced CDMO partner in terms of tech transfer readiness, documentation availability, intellectual property access, and timelines, all ultimately impact cost. They also hinder patients gaining access to treatments as early as possible.

DOI: 10.18609/cgti.2022.166

Our advice is to engage with an expert CDMO that has a broad range of capabilities to support its clients at all stages of clinical development. That means starting from the discovery/proof-of-concept stage, way before preclinical development. The CDMO can then offer support all the way through commercial manufacturing based on its vast experience. While screening for a CDMO partner, ask questions about how they can help de-risk tech transfer whilst ensuring an accelerated path to cGMP manufacturing.

"I can share with you what we are doing here at Thermo Fisher Scientific. Our approach is to provide a comprehensive end-to-end solution under one ecosystem"

Q What can CDMOs do to provide bits of support to biotechs until they are ready?

KW: An experienced CDMO partner can play a consultative role from the beginning, at the ideation stage. Qualified CDMOs have gone through this process many times before for other molecules, so they can ask the right set of questions to stem conversations with innovators and ensure they consider the important developmental aspects as part of their product design.

After all, we cannot design the CMC strategy without first understanding the intended clinical applications. The design of your clinical trials and the design of your manufacturing process have to work hand-in-hand. We need to work backwards to understand the cohort size, the tissue target, the route of administration, the filled volume, the container type, etc. Ultimately, this will all help to shape the decisions surrounding which manufacturing process to use.

If the CDMO is someone like Patheon, for instance, we can also provide our partners with early access to scaled-down cGMP processes for screening molecules before a final candidate is selected.

In summary, if a drug developer is undecided about whether to outsource, or is certain if they will need to leverage a CDMO in the development stage, then start the dialogue as early as possible–ideally, before the preclinical development so that a fit-for-purpose process may be built from the beginning.

Q How are CDMOs helping biotechs solve the challenges of doing business today and also addressing their concerns about the future?

KW: I can share with you what we are doing here at Thermo Fisher Scientific. Our approach is to provide a comprehensive end-to-end solution under one ecosystem. We are the manufacturer of many critical raw materials used for viral vector manufacturing, and

through our newly-launched Patheon Translational Services, we provide molecular biology and viral vector services to generate high-quality, small-scale materials to support early proof-of-concept studies.

Once our partners are ready for pre-clinical development and later stages, we offer process development (PD) and cGMP manufacturing through our pharma services. As I mentioned earlier, you cannot separate clinical trial design from manufacturing, and our organization is in a unique position to also cover clinical research services through our Pharmaceutical Product Development (PPD) team. We also have a robust logistics team to support the transport, storing, and handling of specialized biological samples.

In short, as a CDMO, we help de-risk the tech transfer process, control critical supplies, and simplify business terms such as contracts and licensing agreements–all of which should translate to time and cost savings.

Q What should be considered when choosing a CDMO partner?

KW: There are so many considerations, including the level of transparency and clarity of the CDMO's offering. Some CDMOs offer flexibility to accommodate unique needs, whilst others only provide their platform processes. It is important to understand your biotech's fundamental needs and assess for a good technological fit. If there is limited time and budget available, perhaps there could be an opportunity to partner with a CDMO early to test drive platform technologies without heavy upfront investment.

At Thermo Fisher, we offer our partners access to process technologies and analytics deployed in the cGMP setting, in a scaled down environment. This supports Customers who are going through lead candidate selection but who are not quite ready for the PD and cGMP manufacturing stages yet through our Translational Services offering. There is no commitment to a cGMP contract at this stage, which is important for Customers who want to engage early to evaluate drug candidates and perform proof-of-concept studies.

Another factor to consider is expertise. During your screening process for the right partner, listen to how often the subject of facility availability comes up, rather than other key attributes such as well-trained, talented operators. Do they place emphasis on their team? Ultimately, the operators are going to be supporting your programs. Is there an experienced team of biologists, virologists, bioprocessing engineers, project managers, and clinicians in the organization? Overall, the key is to think on a multidimensional level during your evaluation process.

AFFILIATION

Kim Watanabe, PhD
General Manager & Site Head,
Patheon Translational Services,
Thermo Fisher Scientific

DOI: 10.18609/cgti.2022.166

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

Article source: *This article is based on a Podcast which took place on Aug 18 2022.*

Podcast conducted on: *Aug 18 2022; Revised manuscript received: Sep 23 2022;* ***Publication date:*** *Sep 29 2022.*

De-risk and accelerate the drug development process for gene therapy

Dr Kim Watanabe, General Manager and Site Head for Patheon Translational Services, Thermo Fisher Scientific

In today's challenging financial environment, it is more important than ever to get key biotech business decisions right first time. One of the most important and topical of these is the choice of CDMO partner. Here, Dr. Kim Watanabe, General Manager and Site Head for Patheon Translational Services, a part of the Thermo Fisher Scientific pharma services contract development and manufacturing organizational arm, shares her advice and insights into optimizing outsourcing strategy.

Cell & Gene Therapy Insights 2022; 8(8), 1141 • DOI: 10.18609/cgti.2022.170

PODCAST
PERSPECTIVES

WHAT ARE THE KEY HIGH-LEVEL TRENDS IN THE GENE THERAPY SECTOR THAT ARE IMPACTING BIOTECH DECISION-MAKING AROUND OUTSOURCING?

Lack of harmonization in manufacturing, supply chain continuity issues, a shortage of technical personnel with appropriate experience, and limited regulatory support are all key bottlenecks that most biotech companies looking to outsource are facing. Contract development manufacturing organizations (CDMOs) can offer immediate access to standardized manufacturing processes and analytics, technical expertise and cGMP facilities, regulatory knowledge, and support with scalability.

In our recent experience of the COVID-19 pandemic where the macroeconomy came to a grinding halt, we learned new lessons in drug development. Drug developers are now putting more weight on logistics and raw material inventory management as part of their decision-making process. We will see a greater emphasis on partnering with CDMOs that can offer a true end-to-end solution for support, as a one-stop-shop.

LISTEN TO THE PODAST
or
READ THE ARTICLE

WHEN SHOULD SMALL BIOTECHS ENGAGE WITH CDMOS?

Early engagement and working in collaboration with an experienced CDMO that can support end-to-end manufacturing is key.

Our advice is to engage with an expert CDMO, that have th potential to support customers all along the drug development value chain, including lead candidate screening and selection, process development and cGMP clinical through commercial manufacturing. While screening for a CDMO partner, ask questions on how they can help accelerate the path to cGMP manufacturing.

WHAT CAN CDMOS DO TO PROVIDE SUPPORT TO BIOTECH UNTIL THEY ARE READY?

CDMOs can play a consultative role by asking the right questions upfront to understand the ultimate clinical trial goals and work backwards to design the program.

An experienced CDMO partner can play a consultative role from the beginning at the ideation stage and mitigate the pitfalls found at critical junctions of the drug development process. Qualified CDMOs have gone through this process many times before, for other molecules, so they can ask the right set of questions in moving a drug from concept to clinic ensuring that the manufacturing process can support the intended clinical design. The design of the chemistry, manufacturing, and controls (CMC) strategy works in close conjunction with the manufacturing process and the intended clinical application.

HOW ARE CDMOS HELPING BIOTECHS SOLVE THE CHALLENGES OF DOING BUSINESS TODAY AND ADDRESSING CONCERNS OF THE FUTURE?

CDMOs and small biotechs can develop symbiotic relationships together – 78% of CDMO businesses are made of small emerging biotechs.

As a CDMO, we are hoping to control critical supplies, align the manufacturing platform and analytics from lead identification stage and simplify business terms such as contracts and licensing agreements – all of which should translate to time and cost savings.

WHAT SHOULD BE CONSIDERED WHEN CHOOSING A CDMO PARTNER?

When selecting a CDMO, it is pivotal to focus on a multidisciplinary CDMO, that has technical bioprocessing, regulatory and quality expertise, transparency and flexibility, and access to raw materials to accelerate drug development.

Some CDMOs offer flexibility in process development and analytics to accommodate unique needs of biotech organizations, while others may also provide a standardized platform approach. It is important to understand your biotech's fundamental needs and assess for a good technological fit. If there is limited time and budget available, perhaps there could be an opportunity to partner with a CDMO early to test drive standardized platform technologies without heavy upfront investment in process development.

CELL & GENE THERAPY INSIGHTS

In collaboration with: patheon by Thermo Fisher Scientific

CELL & GENE THERAPY INSIGHTS

CELL & GENE THERAPY MANUFACTURING SCALE-UP/SCALE-OUT

SPOTLIGHT

INNOVATOR INSIGHT

Manufacturing NK cells for the clinic: the Spanish experience

Antonio Pérez-Martínez, Marty Giedlin & Mariam Ammari

This article will discuss a standardized method for manufacturing a high number of clinical-grade NK cells ideal for infusion into patients, expand on the optimization of protocols, and provide a glimpse into the clinical results of infused NK cells at a hospital in Madrid, Spain.

Cell & Gene Therapy Insights 2022; 8(8), 1539–1549

DOI: 10.18609/cgti.2022.225

WHY NATURAL KILLER CELLS?

Natural killer (NK) cells are the main cells in the innate immune system and recognize their targets in a human leukocyte antigen (HLA)-unrestricted manner. This mechanism differs from that of T cells, which bind to specific receptors. NK cell effector function is controlled by a complex array of activating and inhibitory receptors that can differentiate between healthy and stressed cells. It is hypothesized that NK cells recognize their targets by two mechanisms: missing-self recognition, where they attack tumor cells that downregulate the expression of major histocompatibility complex (MHC) class I to evade T cell response, and induced self-recognition, where target cells are recognized due to overexpression of activating ligands that are induced by stress, such as DNA damage or malignant transformation.

These functions are performed in the context of a learning process ('licensing') regulated mainly by inhibitory killer cell immunoglobulin-like receptors (KIR) and their ligands (HLA class I molecules, in humans).

NK CELL ISOLATION

NK cells can be isolated from an initial leukapheresis product by a series of

enrichment steps using CliniMACS Prodigy® devices to achieve 99% NK cells and very few residual T cells (Table 1).

NK CELL THERAPY IN HSCT

In hematopoietic stem cell transplantation (HSCT), – NK cells need to be either alloreactive or activated in order to induce killing of the viral or tumor cells. It has been observed that utilizing alloreactivity in mismatched HSCT for adult and pediatric acute myeloid leukemia (AML patients has resulted in very low incidence of transplant related mortality/graft-versus-host disease and event-free survival of nearly 70%

NK CELLS IN THE CLINIC

Based on previously published data and our own pre-clinical and clinical experience, the use of NK cells to treat cancer patients is safe and feasible. However, the anti-cancer efficacy is limited in extent and duration; around 50% of patients experience remission but this can take 3 months to take effect, and most have begun to relapse within 15 months.

To overcome these limitations, we propose in the future to carry out NK cell engineering to:

1. Improve cytotoxic capacity by creating CAR-NK cells;

TABLE 1

Isolation of NK cells from a leukapheresis product.

n=45	Median	q3	q1	IQR
Patient's age	9.50	12.25	5.00	7.25
Patient's weight	29.00	45.25	21.25	24.00
Donor's age	39.00	44.00	36.00	8.00
Leukapheresis product				
WBC	1.43×10^{10}	1.87×10^{10}	1.24×10^{10}	6.26×10^{9}
NK (%)	7.80	13.00	5.32	7.69
NK cells	1.19×10^{9}	2.18×10^{9}	8.05×10^{8}	1.38×10^{9}
T cells (%)	55.14	67.64	42.86	24.78
T cells	8.02×10^{9}	1.03×10^{10}	6.11×10^{9}	4.14×10^{9}
Viability (%)	99.00	100.00	98.00	2.00
After CD3 depletion				
WBC	6.04×10^{9}	8.44×10^{9}	4.52×10^{9}	3.92×10^{9}
NK (%)	19.57	39.13	10.17	28.97
NK cells	1.44×10^{9}	2.71×10^{9}	5.99×10^{8}	2.11×10^{9}
T cells (%)	0.06	0.13	0.02	0.11
T cells	3.62×10^{6}	8.06×10^{6}	1.13×10^{6}	6.93×10^{6}
Viability (%)	98.00	99.50	98.00	1.50
After CD56 enrichment				
WBC	5.76×10^{8}	1.00×10^{9}	4.35×10^{8}	5.65×10^{8}
NK (%)	99.00	99.33	98.02	1.32
NK cells	5.70×10^{8}	9.92×10^{8}	4.12×10^{8}	5.81×10^{8}
T cells (%)	0.02	0.07	0.01	0.06
T cells	1.40×10^{5}	3.13×10^{5}	3.55×10^{4}	2.77×10^{5}
Viability (%)	100.00	100.00	98.00	2.00
NK cells ($\times10^{6}$)/kg	1.98×10^{7}	3.82×10^{7}	9.53×10^{6}	2.86×10^{7}
T cells ($\times10^{6}$)/kg	2.54×10^{-3}	8.24×10^{3}	0	8.24×10^{3}
Efficiency	50.21	63.90	38.52	25.38

IQR: Interquartile range; NK: Natural killer cells; WBC: White blood cells.

DOI: 10.18609/cgti.2022.225

TABLE 2

Activated and expanded natural killer cells (day +14–19 culture), release criteria.

Test	Specification
Total cell counts	$0.5–20\times10^6$ cells/ml
$CD45^+$ cells viability	≥70%
Phenotype:$CD45^+CD3^-CD56^+$ and $CD45^+CD3^+CD56^+$	≥80%
$CD45^+CD3^+CD56^+$	$\leq10\times10^{10}$/kg
Potency	≥50% versus K562 cell line
Mycoplasma	Negative
Sterility	Sterile blood culture and negative gram
Endotoxins	<0.25 UE/mL
Contaminating cells: K562-mb-IL 15-4BBI	Absence of BCR/ABL (%)

2. Improve delivery into the tumor with chemokine receptor-expressing NK cells;
3. Minimize exhaustion of cells with 'memory phenotype' NK cells.

NK CELL MANUFACTURING

Despite the benefits, NK cells have some important limitations, notably that NK cells represent only a minor fraction of human lymphocytes and large numbers are needed to achieve clinical benefits. These limitations can be overcome by developing good manufacturing practice (GMP) methods for NK expansion, for example using cytokines, different sources of NK cells, or co-culture with irradiated feeder or artificial antigen-presenting cells (aAPCs).

A more than 85-fold NK-cell expansion was reported by Klöss and colleagues in 2017 [1] and we have used a similar process in our laboratory to manufacture two products: IL-15-stimulated NK cells for use in the HSCT setting and activated and expanded NK (NKAE) cells for allogeneic transplantation for sarcoma. Spanish regulators have approved these manufactured cell products for use in patients, with release criteria as shown in Tables 2 & 3.

OPTIMIZING POTENCY & QUALITY OF NK CELL PRODUCT

Culture media

To optimize the potency and quality of NK cell products, different media were compared: RPMI, stem cell growth medium (SCGM), TexMACS, and NKMACS [2]. No significant differences were seen in the numbers of total expanded NK cells, but NKMACS yielded the highest fold increase in NK cells (Figure 1), followed by TexMACS. At the time of this project, NKMACS was approved for research use only, so TexMACS was chosen as the initial culture medium for this work. Subsequently, NKMACS has been approved for GMP use and will be used going forward.

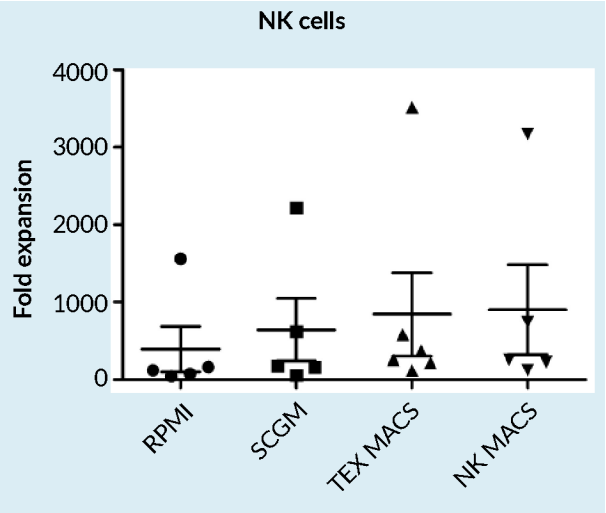
TABLE 3

Overnight IL-15 stimulated NK cells, release criteria.

Test	Specification
Total cell counts	$20–50\times10^6$ /kg
$CD56^+$ cell viability	≥70%
Phenotype: $CD45^+CD3^-CD56^+$	≥75%
T cells $CD45^+CD3^+CD56^-$	$\leq1\times10^4$ cells/kg
Potency	CD107a ≥ 10%
Mycoplasma	Negative
Sterility	Sterile blood culture and negative gram

FIGURE 1

Fold expansion of NK cells with different culture media. Reproduced from [2].



NKMACS and TexMACS media also resulted in the highest purities at day 21 (91 versus 92%, respectively) and the lowest residual T cell rates (3.5 versus 4%). NKMACS and TexMACS also gave the highest percentage of NK dim versus NK bright cells. This is important because bright NK cells lack the KIR CD16$^+$ receptor and are consequently unable to induce antibody-dependent cellular cytotoxicity, leading to a dependence on T cell toxicity.

Starting materials

An important factor in NK cell expansion is the starting material used. Mobilized apheresis yielded fewer NK cells than non-mobilized apheresis and peripheral blood mononuclear cells (PBMCs). After expansion, PBMCs yielded better expansion with IL-21-stimulated compared with IL-15-stimulated cell lines (Figure 2). There was no difference between starting materials in terms of NK receptors or NK cytotoxicity. The chosen starting material was therefore CD56$^+$ PBMCs obtained with non-mobilized apheresis.

Transcriptomic analysis

Transcriptomic analysis of the final products revealed differences between basal NK cells and NKAE cells. There were 2185 differentially expressed genes (1178 upregulated, 1007 downregulated) in NKAE cells. These genes were concentrated in 30 pathways, largely related to cell growth, proliferation, cell death, and metabolism.

Comparing IL-21-stimulated and IL-15-stimulated APCs, more than 600 differentially expressed genes were observed, of which 29 showed upregulation in NKAE cells. Transcriptomic analysis showed that enriched pathways were related to inflammatory and immune system responses.

Comparing PBMC with CD45RA$^+$ cells as starting material, 37 genes were upregulated, and 11 genes were downregulated in PBMC-derived NKAEs versus CD45RA$^+$-derived NKAEs. Two pathways were enriched, associated with hematopoietic cell lineage and metabolism of arachidonic acid.

GMP manufacturing

NK cells used by University Hospital La Paz were manufactured in a GMP facility, using an automated activation expansion process performed with the CliniMACS Prodigy instrument. The CliniMACS T520 tubing set and T cell transduction protocol were used.

At day 0, the co-culture was initiated by using 2×10^6–2.5×10^6 NK cells and 4×10^7 K562mbIL15 or K562mbIL21 cells previously irradiated with 100 Gy. Cells were cultured in 70 mL of GMP-grade TexMACs medium supplemented with 5% human AB serum (Sigma) and 100 IU/mL of IL-2 (Miltenyi Biotec). NK cells were incubated in the culture chamber (37°C and 5% CO2) in a static culture for the first week. At day +7, agitation was started, and 70 mL of fresh complete medium was added to the culture. Cells were expanded for 14 days before being harvested.

DOI: 10.18609/cgti.2022.225

Sampling was performed at day +7 for process controls, including cell counts, viability, $CD56^+/CD3^-$ cell content, mycoplasma, and sterility. When the expansion was complete, cells were automatically collected in 0.9% sodium chloride solution supplemented with 0.5% human serum albumin (Albutein 20%, Grifols, Barcelona, Spain), in a sterile bag. Release quality controls included total cell counts, viability, $CD56^+/CD3^-$, $CD3^+/CD56^-$and $CD56^+/CD3^+$ cell content, cytotoxicity against K562 cells, Gram staining, endotoxins, cell impurities (K562mbIL15 or K562 mbIL21), mycoplasma, and sterility.

The acceptance criteria included viability higher than 70%, cytotoxicity against K562 higher than 50% at a ratio of 1:8 for effector and target cells, Mycoplasma spp negativity, sterility (zero-colony forming units), endotoxins less than 0.25 EU/mL, and undetectable BCR-ABL.

CONCLUSION

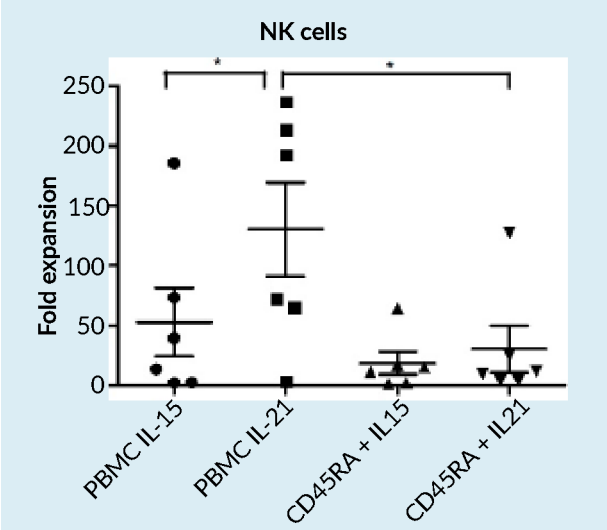
An optimized protocol is described to obtain NKAE cells by using four different culture growth media (RPMI, SCGM, TexMACs, and NKMACs) and two different NK cell sources [PBMC or $CD45RA^+$ cells and two distinct irradiated aAPC (K562mbIL15 or K562mbIL21)]. TexMACs was determined to be the most suitable cell culture medium to expand NK cells (although this has now been replaced with NKMACS).

NK cells could be activated and expanded from $CD45RA^+$ cells obtained from non-mobilized apheresis, although the use of PBMC as the NK cell source yielded the highest numbers of purified NKAE cells. When K562mbIL21 was chosen as the APC, the highest numbers of NKAE cells and lowest T cell contamination were achieved regardless of the NK cell source used. All NKAE cells obtained from either PBMC or $CD45RA^+$ expanded with K562mbIL15 or K562mbIL21 showed comparable antitumor ability against sarcoma, T-ALL, CML, neuroblastoma, and rhabdomyosarcoma cells.

Finally, clinical manufacturing of NKAE cells was fulfilled in an automated closed system CliniMACS Prodigy by using $CD56^+$ cells and either irradiated K562mbIL15 or K562mbIL21. In both processes, sufficient numbers of NKAE cells with high purity and low T cell contamination were manufactured after 14 days in culture. The release tests showed that manufactured NKAE cells met the requirements and specifications from the regulatory agency and thus, were suitable for clinical use. The NKAE cells manufactured are suitable for direct infusion to the patient or cryopreservation – and could also serve as a platform for more advanced NK cell therapies such as a combination with bi-specific killer engagers (BiKEs) or genetic modification to express chimeric antigen receptors (CARs).

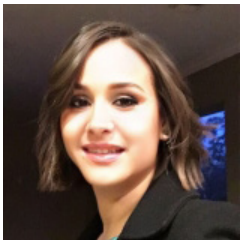
FIGURE 2

Fold expansion of NK cells with different starting materials. Reproduced from [2].



ASK THE EXPERTS





Charlotte Barker, Editor, BioInsights speaks to (pictured left to right) **Marty Giedlin, Senti Bio**, **Antonio Perez-Martinez, La Paz University Hospital, Madrid** and **Mariam Ammari, MD Anderson Cancer Center** answer your questions on NK cell manufacturing.

Q How big an impact does the starting material have on the final product?

MG: Based on Antonio's experiences and the experience at Senti in developing an alloreactive NK CAR, the starting material from healthy donors is critical. Our screening paradigm first and foremost includes manufacturability – whether we can make the dose that we plan to put into phase 1. We also consider whether the cells that we make are functional and able to provide a therapeutic effect for the patient post-infusion. We spend a lot of time characterizing our starting material and trying to come up with algorithms that will show us what fits our process, and then we recall suitable donors for GMP apheresis to start making cell banks for manufacturing.

AM-P: I agree with Marty. The starting material is important. The most important learning point from our experience was not to use mobilized apheresis because it yields fewer NK cells and they do not expand as well as those obtained with non-mobilized apheresis.

MA: I agree with both Marty and Antonio that the starting material makes a big difference in the final product. In our case, we usually begin with a frozen starting material like umbilical cord blood. We found out that how the product is cryopreserved before activation and expansion makes a big difference to the final product. NK cells survive for only 2 weeks, so we need to understand the starting material and the quality of our NK in the starting material, and how that might affect the final product, the expansion, and also the exhaustion of NK cells after they are infused into patients.

DOI: 10.18609/cgti.2022.225

Q What are the most important criteria to consider when selecting donors and what screening methods and approaches should be used?

MA: For manufacturing products, the criteria for the starting material should be the viability, the age of the donor (if starting from PBMCs), the type of disease, and whether the product is autologous. We take a medical history and perform serology for infectious diseases to see what the impact of the starting material will be on the manufacturing process and the final product.

AP-M: Initially, we were worried about how to select the best donor and tried to look for donors with the best KIR haplotypes or alloreactive NK cells. However, helpful reviewers of our work pointed out that it is unnecessary to use alloreactive NK cells to expand because the cells are going to be modified and the phenotype is going to change. So, the most important thing when selecting a donor of NK cells for expansion is to choose a young, healthy donor, as Mariam said. Of course, if you are not going to expand the cells and plan to infuse fresh NK cells without any modifications in a haploidentical setting, you should try to select alloreactive NK cells from a KIR-matched donor.

MG: I agree with both Antonio and Mariam. Expansion is extremely important in manufacturing, but it is also important to measure that against what is 'left in the tank' post-infusion: is there still some replicative capacity of the NK cells that can seek and kill tumors? Therefore, we measure both aspects and try to set up screening so that we have the appropriate time of expansion – post-activation transduction in our case – and still some life left in the cells afterward. Part of our small-scale screening operation aims to identify donors who are most applicable to the process.

Q What are the biggest challenges when considering the manufacturing of NK cells in a hospital setting, specifically?

MA: Based on my experience working in GMP manufacturing in a hospital setting, I think the most important challenge for hospitals is insufficient funds and not being up to date with the technology and equipment used in commercial manufacturing. Also, there are limited pharmaceutical investments in long-term projects with hospitals. If big pharmaceutical companies invested in hospital settings, it would help to make cell therapy accessible for more patients in more hospitals.

AP-M: Our institution in Spain is a public hospital, so the main challenge is human resources. When trying to incorporate a new strategy in cell transplantation or new cell therapy, first you have to convince your colleagues and your group. Second, you need human resources and facilities to perform the processes. In Spain, this would normally be performed in a research hospital but there are very few. It would be helpful to incorporate researchers in university hospital departments.

Q Could the panel comment on novel bioprocessing technologies with the potential to reduce manufacturing timeframes and requirements for technical expertise?

AP-M: Many of the processes remain very work-intensive. More closed and systematic ways of working will reduce the demand for human resources and make results more repeatable. Of the novel bioprocessing technologies, CliniMACS Prodigy has helped us to make more homogenous products across different locations using fewer resources.

MA: I agree with Antonio. Having a closed system that decreases human resource demands, improves reproducibility, and makes the manufacturing process scalable and functional will be great for the future of cell therapy.

MG: I think the other area to focus on is analytics. Particularly what is being termed 'integrated analytics.' As well as closed systems, systems that continuously monitor viability, metabolic state, residuals, etc. in cell culture will be very helpful. Ideally, those systems would be built-in and reactive; for example, sending notifications to your cell phone that a certain parameter has fallen out of range but has been automatically corrected. That would help to make better cultures but also to take the pain out of constant sampling and eliminate a lot of 'touches' to your cultures going forward.

Q What are the best methods for assessing the purity and potency of NK cells?

AP-M: Not necessarily the best method but probably the fastest, is a panel flow cytometry to check the purity of cells. To check the potency, you can use decannulation by flow cytometry or any functional test against different cell lines. In my opinion, if you are going to use your expanded cells against a solid tumor, these functional tests should be against cell lines similar to the target cells. However, this is not easy.

MG: One thing that is becoming more acceptable, or maybe more characteristic, is the use of single-cell assays. In the NK field, you're starting with only a few cells, and your expansions are still somewhat limited, so you need as many cells as possible for the patient. For potency in particular, there are systems available that look at single-cell RNA expression. There are also ways to assess potency by looking at pathways or what your cell can do after activation. I am looking forward to getting beyond the two-dimensional mast cell assays that have been used for the past 25–30 years, and into something more indicative of true potency (e.g., whether NK cells can find and kill the tumor). Maybe some of the three-dimensional models that are emerging for assessing trafficking and killing will provide another way of looking at the potency of NKs.

MA: Similar to Antonio and Marty, we use flow cytometry to assess the purity of our CAR NK cells, but we also have some cytotoxicity assays that we developed

DOI: 10.18609/cgti.2022.225

in-house. I also agree that we need more single-cell sequencing technologies to be integrated into our process.

Q When it comes to ensuring the quality and compliance of your final drug product, what are the key considerations and approaches?

MA: When it comes to ensuring final product quality, we measure safety and we have some release assays for sterility and Mycoplasma spp, but, since we use virus transduction, we do replication-competent retrovirus testing. We also measure vector copy number, and we assess the transduction by flow cytometry.

MG: We are inserting CARs and other genes into our NKs as a way of turning them into 'mini-computers', so we have to show activity for up to four different genes. We must ensure that copy numbers are within the U.S. Food and Drug Administration (FDA) ranges and assess the transduction efficiency and potency of the end product. We also have irradiated feeders that have been genetically manipulated and we use a polymerase chain reaction and a flow cytometry assay to show that they are not present in the final product. Last, but not least, because we are making bags of frozen NKs from healthy donors, these must be extensively tested for viruses according to FDA regulations. There is a lot of testing going on for these allogeneic products that may have not been necessary for an autologous approach, but certainly, the FDA is very interested in making sure that we're not transmitting any viruses or introducing any oncologic events via our transduction techniques.

REFERENCES

1. S Klöß, O Oberschmidt, M Morgan *et al.* Optimization of human NK cell manufacturing: fully automated separation, improved ex vivo expansion using IL-21 with autologous feeder cells, and generation of anti-CD123-CAR-expressing effector cells. *Hum. Gene Ther.* 2017; 28(10), 897–913.
2. A Fernández, A Navarro-Zapata, A Escudero *et al.* Optimizing the procedure to manufacture clinical-grade NK cells for adoptive immunotherapy. *Cancers (Basel).* 2021; 13(3), 577.

AFFILIATIONS

Antonio Pérez-Martínez, MD & PhD
Head of Pediatric Hemato-Oncology and Hematopoietic Transplant Service,
La Paz University Hospital, Madrid

Marty Giedlin
Senti Bio

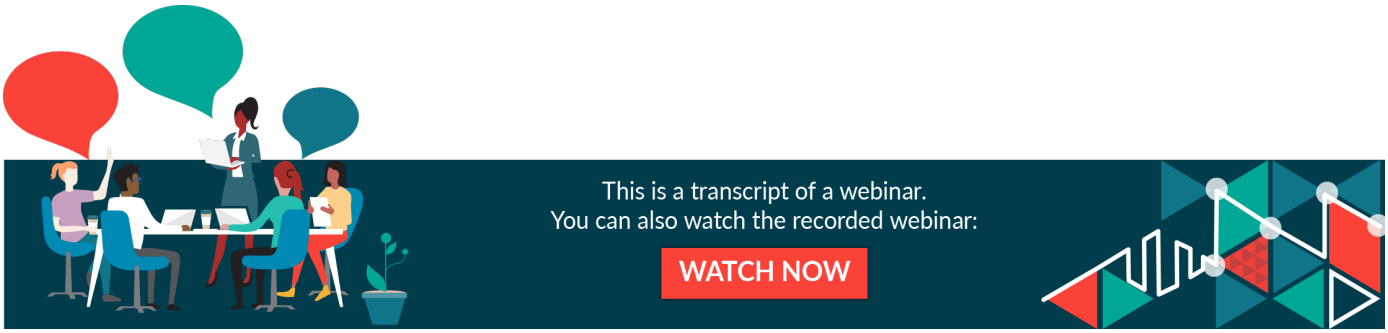
Mariam Ammari
MD Anderson Cancer Center

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *The named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*
Acknowledgements: *None.*
Disclosure and potential conflicts of interest: *Marty Giedlin is a Senti BioSciences, Inc. employee and stockholder.*
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: Sep 27 2022; **Revised manuscript received:** Dec 5 2022; **Publication date:** Dec 14 2022.



DOI: 10.18609/cgti.2022.225

Miltenyi Biotec

The CliniMACS Prodigy®

Mastering the complexity of cell processing

- A single closed system from starting material to final cellular product
- Full automation of complex procedures, including manufacturing of gene-engineered T cells
- Integrated electroporation system for non-viral gene delivery
- Multiple sampling pouches for convenient IPC/QC

▸ miltenyibiotec.com

CELL & GENE THERAPY INSIGHTS

CELL & GENE THERAPY MANUFACTURING
SCALE-UP/SCALE-OUT

SPOTLIGHT

LIVE30 TRANSCRIPT

Achieve significantly increased adenovirus yield with density gradient ultracentrifugation: a comparative study

Shawn Sternisha

Adenoviral vectors are highly effective tools for gene therapy due to their high gene transduction efficiencies, safety, and tunability. Although numerous technologies exist for the downstream purification of adenovirus, several critical manufacturing challenges still exist, including scalability, low recovery, and the removal of impurities. Two of the most prominent adenovirus purification techniques include density gradient ultracentrifugation (DGUC) and ion exchange chromatography (IEC). Although IEC is well established as a scalable approach to biomolecule purification with short separation times, it suffers from several limitations in the context of adenoviruses, including low binding capacity, serotype dependency, and a trade-off between yield and purity. DGUC, however, is a robust, serotype-independent method that offers improved product recovery by exploiting subtle differences in the buoyant density between full particles and process impurities.
In this article, both techniques are directly compared, and significant improvements in product critical quality attributes (CQAs) including more than 200-fold increases in yield and concentration are observed for the DGUC-purified AdV5. In addition, universally applicable approaches to maximize throughput and efficiency when scaling up or down adenovirus production are explored.

Cell & Gene Therapy Insights 2022; 8(8), 1257–1266

DOI: 10.18609/cgti.2022.184

Within the field of viral vectors, there are several different therapeutic mechanisms that are exploited. Vectors are used as delivery vehicles in gene therapy, as viral vaccines to deliver genetic material to provide immunity, and as replication-competent viruses in oncolytic virotherapy. Within gene therapy, adeno-associated virus (AAV), adenovirus and lentivirus are all promising vectors for clinical applications.

Nearly half of all gene therapy trials on the Wiley database employ adenovirus. Adenoviruses have the advantage of large size, with a diameter of ~90 nm, providing a large packaging capacity for ~8kb of double-stranded DNA. They also have a high transduction efficiency with a variety of cell types. They are non-integrating vectors, meaning they do not integrate their genetic material into the genome of the patient. Additionally, there is a wide diversity of seven serotype subgroups composed of over 50 different serotypes, enabling high tissue tropism.

With the increasing demand for gene therapies, producing enough viral vectors is a key challenge throughout development. Despite steady innovations in both the upstream and downstream workflows, supply has not kept up with demand. This will likely continue as larger clinical studies are conducted and more prevalent diseases are targeted. Both the number of patients and the required dosing regimens are factors that contribute to the demand for viral vectors.

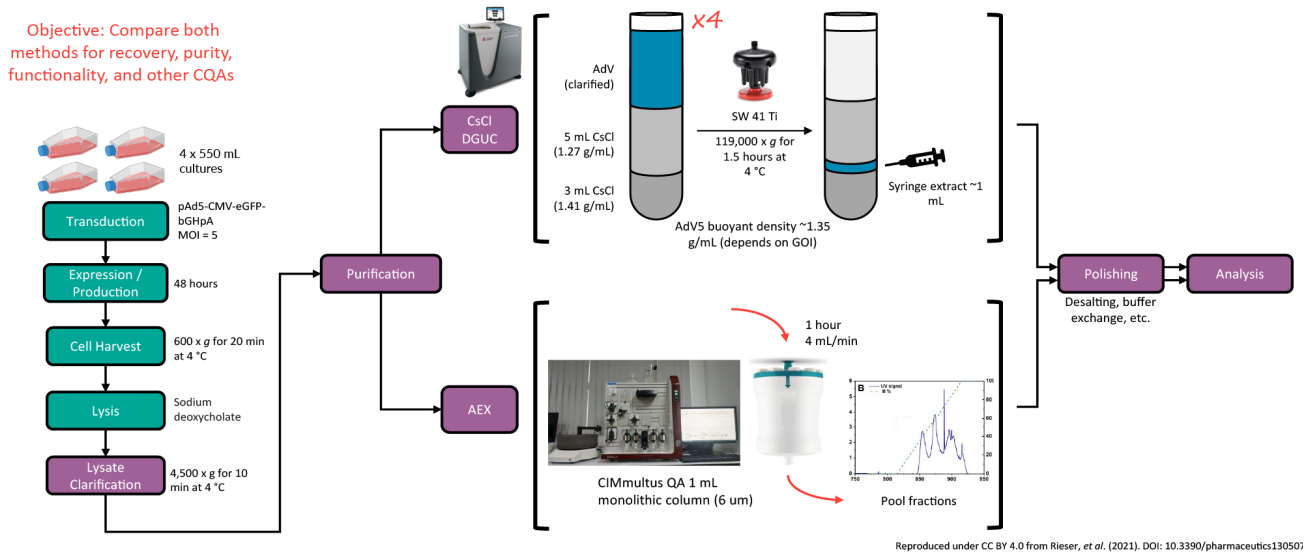
For example, there are roughly 2000 new cases of spinal muscular atrophy (SMA) per year, and treating all of these patients with Zolgensma translates to a requirement of 42000 L of cell culture. There is also an immense manufacturing burden for the treatment of amyotrophic lateral sclerosis (ALS), with a requirement of nearly 500000 L of cell culture. While scale is a critical component of meeting these demands, process yield should not be overlooked.

DOWNSTREAM PURIFICATION OF ADENOVIRUS

In the workflow of a generic viral vector manufacturing process, a cell bank is first expanded. The rest of the upstream workflow includes infection and subsequent production of viral particles, followed by cell harvest and lysis. The lysate is clarified and the virus particle is purified before formulation and vial fill. Adenovirus is prone to inefficient packaging

FIGURE 1

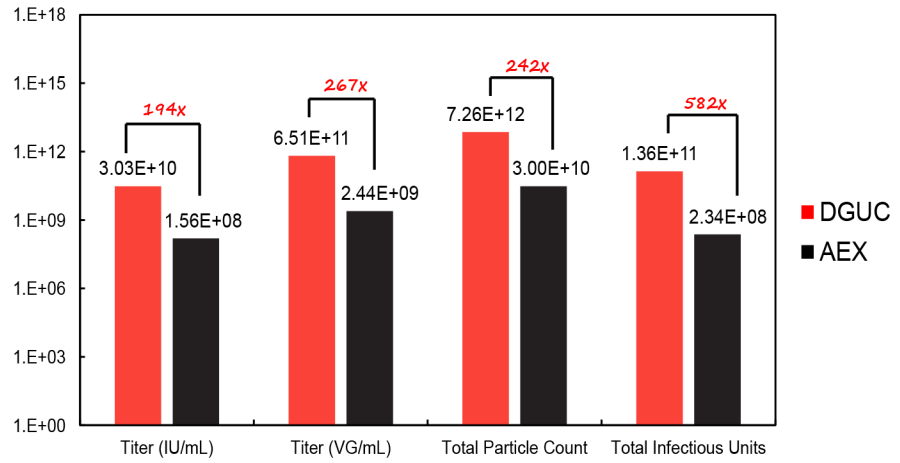
Experimental process for AdV5 purification using DGUC versus AEX.



DOI: 10.18609/cgti.2022.184

FIGURE 2

Performance data for titer, total particle count, and total infectious units for AdV5 purified via DGUC versus AEX.



Purification Method	Final Product Volume (mL)	InfectiousParticles/ Total Particles
DGUC	4.5	0.019
AEX	1.5	0.0078

of DNA cargo, which leads to empty and full particles. Therefore, in downstream purification, enrichment for full particles is an essential objective.

DGUC is a high-resolution purification method that separates particles based on their buoyant density. A defined amount of a density gradient-forming material, such as cesium chloride (CsCl), is added to the tube and spun. After some time, a density gradient forms and the particles of interest migrate to the position where their density is equal to the surrounding media (isopycnic point). In the density gradient, band position directly correlates to particle fullness, meaning denser (fuller) particles will sit lower in a density gradient. DGUC offers high purity separation and extremely high yields in a serotype-agnostic process. Protocols can be directly translated from one serotype to the next, and multiple serotypes can be purified in a single run.

Anion exchange chromatography (AEX) is another popular option for purifying adenovirus. AEX separates capsids based on charge. Charge differences in isoelectric point arise from the presence or absence of the DNA cargo. The AEX resin is functionalized with cations that bind adenovirus. After washing, elution is achieved by flushing the column with a buffer containing high concentrations of counter anions, which displace the virus. AEX is a highly scalable and automatable process.

CASE STUDY: QUALITY & YIELD OF ADV5 PURIFIED VIA DGUC VERSUS AEX

In this study, the primary objective was to compare DGUC and AEX for particle recovery and yield, functionality or infectivity, and other critical quality attributes (CQAs). The human adenovirus type 5 (AdV5) purification processes used either AEX or DGUC, as outlined in Figure 1. Yield was quantified using immunohistochemical detection, ddPCR, and OD260 and the results are shown in Figure 2.

This study found that DGUC yields significantly more AdV5 than AEX. DGUC provides a 582-fold increase in the number of infectious particles recovered, as well as a higher ratio of infectious particles to total particles.

DGUC was also shown to give rise to more infective adenovirus particles and improved endotoxin removal. DGUC produced adenovirus particles with ~28% higher functionality and was more efficient at removing endotoxin even at a higher viral concentration.

With both purification schemes, no bacteria or mycoplasma contaminants were

detected in either sample. However, endotoxin was detected at 1.1 units per mL in the AEX sample, whilst undetectable in DGUC. Residual host cell proteins were detected in the DGUC sample, but when the sample was diluted to the same adenovirus concentration as the AEX sample, the level was well below the limit of detection.

DGE-AUC CHARACTERIZATION

The samples were also analyzed using density gradient equilibrium analytical ultracentrifugation (DGE-AUC). In a manner analogous to preparative DGUC, DGE-AUC employs CsCl to form a gradient and separate particles according to their buoyant density.

Absorbance and interference optics in Beckman Coulter's AUC instrument, the Optima AUC, allows the monitoring of gradient formation and directly measures the signal at radial positions once equilibrium is achieved. Using a six-sector centerpiece, it is possible to run up to 21 samples simultaneously and achieve equilibrium in 1 h. The chosen run speed affects the slope and the range of the gradient that forms. Higher speeds tend to offer higher sensitivity, while lower speeds favor better resolution.

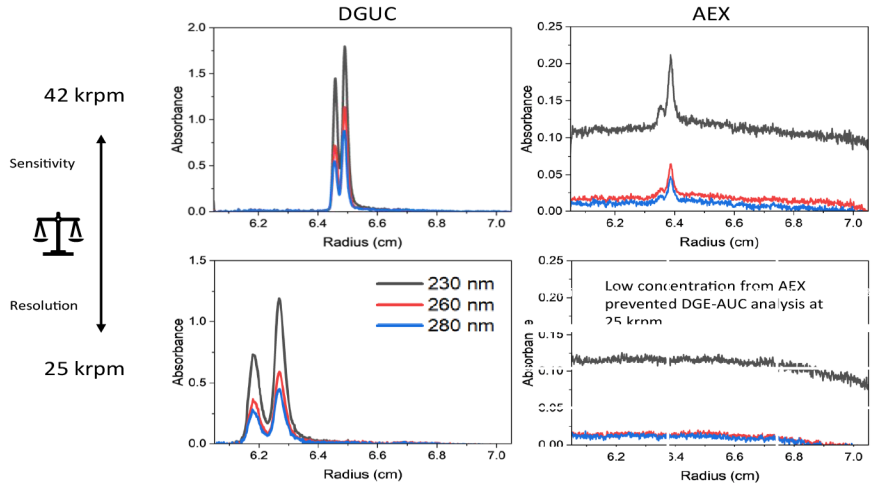
As shown in Figure 3, higher intensity peaks were observed at 42 krpm while near baseline resolution was achieved by dropping the speed to 25 krpm for the DGUC-purified sample. Using DGE-AUC, two peaks were observed in each sample, and the 260/ 280 ratios were around 1.3, within the same range as full particles.

OPTIMIZING DGUC

The protocol for DGUC previously discussed is a rapid swinging-bucket protocol, which takes around 1.5 h to complete in the SW 41 Ti. In this protocol, the result is a step gradient rather than a continuous gradient.

FIGURE 3

DGE-AUC characterization of vector purified using DGUC versus AEX.

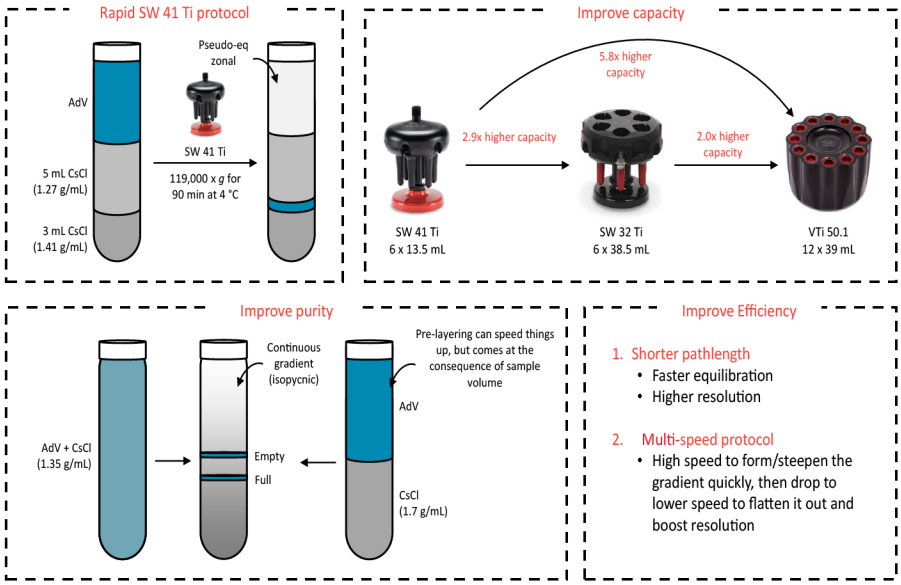


Purification Method	Peak 1 260/280	Peak 2 260/280
DGUC (42krpm)	1.26	1.30
AEX (42krpm)	1.15	1.16
DGUC (25krpm)	1.29	1.29
AEX (25krpm)	NA	NA

DOI: 10.18609/cgti.2022.184

FIGURE 4

Optimization of DGUC through logical and predictable changes.



To improve a workflow with DGUC (Figure 4), the first aspect to consider is improving capacity. By switching to another swinging-bucket option, the SW 32 Ti, a 2.9-fold higher capacity can be achieved. In addition, by switching to Beckman Coulter's newest vertical rotor, the VTI 50.1, an almost 6-fold increase in capacity can be further achieved.

Another approach to improve workflow is to improve purity via continuous/linear density gradients. In contrast to the equilibrium-zonal purification using the SW 41 Ti protocol, formation of continuous gradients provides enhanced resolution. CsCl can be added directly to clarified lysate as a solid or it can also be layered with the sample on top to significantly reduce run time. However, there is usually a trade-off with sample volume in this case.

Other methods of improving DGUC efficiency include using a shorter pathlength, which allows for faster equilibration and higher resolution. This means that the vertical rotor is the best option for density gradient separations. Multi-speed protocols can also be implemented to reduce the time required to reach equilibrium.

Beckman Coulter Life Sciences offers a wide range of ultracentrifugation tubes from 2–100 mL, with the 39 mL tubes being the most popular option. Most tubes are available in a variety of formats including open top, Quick-Seal (permanent seal) and OptiSeal (plug seal). Different tube materials including polypropylene and UltraClear are also available.

TRANSLATION INSIGHT

In a case study comparing DGUC and AEX, a single run in a low-capacity swinging-bucket rotor could generate over $1.09x10^{13}$ viral particles for adenovirus. Using this AEX purification protocol, 110 L of cell culture would be needed to achieve the same yield. DGUC offers a high yield and concentration of functional particles while minimizing other contaminants. This process is most efficient in a vertical rotor, which are available in a broad range of sizes to support analytical or Design of Experiment (DoE) needs as well as large-scale preparative workflows.

FIGURE 5

Scaling up and scaling down DGUC.

Q&A

David McCall, Editor, *BioInsights* speaks to (pictured) **Shawn Sternisha,** Senior Field Applications Scientist, Biotechnology Business Unit,Beckman Coulter Life Sciences

Q What are the main considerations for changing from the swinging-bucket or fixed-angle to a vertical rotor?

SS: Swinging bucket has the longest pathlength, while the vertical rotor has the shortest. The fixed-angle rotors are somewhere in the middle. The short pathlength allows you to reach equilibrium significantly faster if you are running a density gradient.

In addition, our VTi 50.1 rotor has a nominal capacity of 468 mL, making it the largest vertical rotor on the market by about 50%. If you are doing step or continuous gradients on a large scale, this is the best option.

DOI: 10.18609/cgti.2022.184

The caveat with vertical rotors is that they are only good at isopycnic density gradient. For labs with a variety of different applications including gradients, pelleting, and flotation, a fixed-angle rotor can provide the additional versatility.

Q Can vertical rotors be used for iodixanol gradients, and what other uses do they have?

SS: We have a significant number of customers who do use iodixanol and vertical rotors, at different scales. In this case, it does not matter if it is a step or a continuous gradient. Either one should be amenable to a vertical rotor, which has the highest efficiency for those types of purifications.

For vertical rotors, density gradients are their primary use and they are by far the best option. Fixed angles are best for versatility. The swinging bucket is the best choice if you plan on doing rate zonal experiments, which is a pelleting experiment through a gradient. The long pathlength is good for high resolution in those cases.

Q Have you ever tried adding CsCl in clarified lysate, and then DGUC without layering the two CsCl gradients?

SS: This is something that we have explored through a different collaboration and it should be possible. You would need to make sure that you have a sufficient concentration of your viral vector to ensure you can see the band at the end, although it may be possible to use fractionation instead of extracting bands.

Q What do you suggest using for the polishing step after the ultracentrifugation?

SS: The removal of CsCl can be done in a variety of ways, dependent on your needs and what volume you are working at.

At the lower scale, spin concentrators are very common. Our sister company Pall offers some that we have used in the lab ourselves. There are also de-salting columns, and at a larger scale, tangential flow filtration is another popular option for polishing.

REGULATORY / TRADEMARK STATEMENTS

INTELLECTUAL PROPERTY

AFFILIATION

Shawn Sternisha
Senior Field Applications Scientist,
Biotechnology Business Unit,
Beckman Coulter Life Sciences

DOI: 10.18609/cgti.2022.184

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *The named author takes responsibility for the integrity of the work as a whole, and has 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 author 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: *Sep 13 2022****; Revised manuscript received:*** *Oct 17 2022****; Publication date:*** *Oct 20 2022.*

CELL & GENE THERAPY INSIGHTS

CELL & GENE THERAPY MANUFACTURING SCALE-UP/SCALE-OUT

SPOTLIGHT

INTERVIEW

Collaboration, technology & innovation in the scale-up of viral vector manufacturing

Abigail Pinchbeck, Assistant Editor, *BioInsights*, talks to **Matthias Hebben** PhD, VP & Head of Technology Development, LogicBio Therapeutics

MATTHIAS HEBBEN has been serving as vice president of technology development at LogicBio Therapeutics since February 2019. In his role, he is leading the CMC efforts, including vector core, capsid optimization, process development, analytical development and clinical product manufacturing. Before that, he served as director of technology development and head of bioprocess development at Genethon for 6 years. Before that, he occupied several positions at Vivalis (Valneva), Intervet Shering Plough and Virbac. Matthias has a PhD in molecular biology and a MSc in bioprocess engineering.

Cell & Gene Therapy Insights 2022; 8(8), 961–965

DOI: 10.18609/cgti.2022.142

Q What are you working on right now?

> "The challenges for transfection scale-up are mainly to keep the transfection efficiency consistent."

MH: Currently, we are pursuing our clinical trial, SUNRISE, which assesses our GeneRide® genome editing technology in patients suffering from methylmalonic acidemia (MMA). In parallel, our discovery team is developing GeneRide and gene therapy products for other metabolic disorders.

On the chemistry, manufacturing, and controls (CMC) side, my team is improving our manufacturing platform for our adeno-associated virus (AAV) products. We have developed proprietary plasmids and improved the transfection conditions in suspension HEK293 cells. The combined technology, called mAAVRx™, has shown a significant increase in our vector yields of up 30-fold higher than what we used to see with our first-generation process.

We are also actively working on polishing methods to enrich full capsids in our drug products. The goal is to develop a scalable method, and here our process development team has been very successful with a chromatography method leading to more than 80% of full capsids in the drug product.

Q What are the current most pressing issues in the scalability and large-scale manufacturing of viral vectors?

MH: Transient transfection is still the most popular method for AAV manufacturing. The challenges for transfection scale-up are mainly to keep the transfection efficiency consistent. Transfection is a highly complex process that requires tight control of the DNA complexation time as this governs the size of the DNA particulates. At a large scale, every operation takes more time, which becomes a problem for the time-sensitive transfection step. If this step is not perfectly under control, the vector yields may be decreased, and the quality attributes could be impacted.

Besides the upstream process, there are multiple filtration and chromatography technologies that are highly efficient in the purification of AAV vectors. Therefore, I see fewer challenges in scaling up downstream processes.

Q LogicBio® recently entered an industrial-scale AAV manufacturing collaboration with Exothera and Polyplus. What are the key goals of this collaboration?

MH: The collaboration between Exothera, LogicBio, and Polyplus was established with the aim to assess the scale-up of a transient transfection-based AAV

DOI: 10.18609/cgti.2022.142

manufacturing process in suspension cells. Each member of the consortium will bring their expertise in process scale-up, AAV manufacturing, and transfection technology. The project aims to assess the reproducibility and the comparability between different scales of suspension bioreactors, up to 2000 L. In this partnership, LogicBio will have the opportunity to assess mAAVRx technology at a large scale. It is an exciting project because we expect the results to provide valuable proof of concept for each partner's asset: 2000-L scale manufacturing capabilities for Exothera, scalability of mAAVRx platform for LogicBio, and large-scale efficacy of FectoVir-AAV for Polyplus.

Q With an increase in vector manufacturing facilities designed for >2000 L production capacities, what technological solutions are required to enable these industrial scales?

MH: Everyone is hoping for stable cell lines, which would allow the removal of transfection. However, generating a stable cell line for viral vectors is much more complex than it is for monoclonal antibodies. First, there are multiple genes to be integrated and expressed at specific levels relative to each other. Second, some of the viral proteins are toxic to cells, which may require the use of inducible promoters. Consequently, cell line development can be a lengthy process, while most of the time, companies set up aggressive timelines to Phase 1 clinical trials.

For this reason, I believe the transfection process will certainly remain a viable option for many years, including for commercial phase manufacturing. To allow this, the field will need highly efficient transfection reagents and standardized procedures to ensure consistent transfection conditions.

Process economics is also a challenge to be addressed. The raw materials for gene therapy are extremely costly, particularly GMP-grade plasmids. It would be interesting to see how we could decrease these costs in the future.

Q You have over 20 years of scientific and technical experience – can you reflect upon that long journey, and share your key concerns as you look at the viral vector field today?

MH: When I started my career more than 20 years ago, I was using the Vaccinia virus as a vector to make a new generation of vaccines. At that time, there was excitement around a non-replicative Vaccinia strain called Modified vaccinia Ankara (MVA). After many years of development, the field realized that the process yields were low, making these vaccines more expensive than the conventional ones. In addition, the duration of immunity with MVA vectors appeared to be rather short. MVA vectors progressively fell off people's radars.

Today, the gene therapy field is facing similar challenges: low yields, high manufacturing costs, and the concern that AAV vectors may not persist for the whole patient's lifespan, at

least for liver-targeting AAV therapies. In addition, multiple cases of safety issues and serious adverse events have been observed with various AAV products in the past few years. All these factors have recently raised concerns and mistrust in the Gene Therapy field.

I think there is an urgent need to solve these problems. LogicBio has built three development axes to address these questions. Our GeneRide technology is designed to integrate the therapeutic gene in a specific locus of the patient's genome, with the goal of making the treatment durable. It does not include a promoter, providing a potential safety benefit. In addition, our mAAVRx platform has shown an increase in the manufacturing yield of AAV vectors by around 15 to 30-fold. Finally, our sAAVy™ capsid engineering platform is designed to generate novel AAV capsids that are effective at lower doses, a key factor to improve product safety and confer manufacturing benefits like cost of goods improvements.

There is still a long road ahead for the gene therapy field, but so many great results have been achieved so far, which makes me hopeful that the scientific community will maintain momentum and continue improving the technology at a fast pace.

Q What further improvements or innovation would you like to see in large-scale viral vector manufacturing in both the near and far future?

MH: The field would benefit from a better understanding of AAV production mechanisms at the cellular and molecular levels. In line Process Analytics Technologies (PAT), like metabolic monitoring, real-time quantification of viral particles, and full to empty capsid ratios during cell culture, would help to characterize better the processes, which might then open new paths for process optimization.

Returning to the transfection challenges at a large scale, I think it would be interesting to have equipment vendors proposing technical solutions and devices to support DNA mixing and complexing in large containers as well as easy and fast transfer into the bioreactors.

Q What are your key goals and priorities both for yourself and for LogicBio as a whole over the next 1–2 years?

MH: The gene therapy field and the biotech world, in general, are facing an unfavorable economic context. The best approach in these difficult times is to focus on innovation to develop better treatments at lower costs. The first commercially approved gene therapies are so expensive that the current economic model may not be sustainable, especially in this new period of financial crisis. LogicBio remains strongly committed to

"The field would benefit from a better understanding of AAV production mechanisms at the cellular and molecular levels."

DOI: 10.18609/cgti.2022.142

patients' health and accessibility to treatments. In this context, I will continue to lead the efforts toward the development of more efficient and cost-effective manufacturing processes as well as the engineering of more potent capsid serotypes. Ultimately, we hope to implement a universal manufacturing platform to shorten the development timelines of our next products.

AFFILIATION

Matthias Hebben, PhD
Vice President of Technology Development,
LogicBio Therapeutics

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 patents planned/issued/pending GeneRide, sAAVy and mAAVRx technology. The author has stock options of LogicBio.*

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 based on an interview with Matthias Hebben carried out on Aug 5 2022.*

Interview held: *Aug 5 2022*; ***Revised manuscript received:*** *Aug 12 2022*; ***Publication date:*** *Sep 2 2022.*

CELL & GENE THERAPY INSIGHTS

CELL & GENE THERAPY MANUFACTURING
SCALE-UP/SCALE-OUT

SPOTLIGHT

LIVE30 TRANSCRIPT

Optimizing *E. coli* cell growth performance with in-line, real-time OD600 (abs/mm) monitoring during the fermentation process

Tanja Buch & Ramsey Shanbaky

The increasing demand for cell and gene therapies (CGT) has created a need to optimize manufacturing process steps using Process Analytical Technologies (PAT) to lower costs and increase yields. Plasmid DNA (pDNA) is a precursor to the development of CGTs and is met with many challenges throughout the production process due its size, viscosity, and similarities to other impurities. The generation of these plasmids in high density *E. coli* cultures can be difficult to monitor using traditional off-line OD600 methods due to the careful sample preparation, fast growth rate, and dilutions required to be in the linear range of a standard spectrophotometer. This study proposes utilizing an in-line, variable pathlength spectrophotometer in a recirculation loop during fermentation to monitor and optimize the growth of E. coli cells in real-time. This provides distinct advantages over traditional methods due to the constant modification of path lengths to create a slope-based solution that is always within the linear range of the equipment, with no buffer correction required. The growth curve is tracked in real-time using the slope (Abs/mm) at 600 nm to measure cell density and to optimize growth performance of cells. This will serve as a proof-of-concept for the optimal cultivation of *E. coli* and production of pDNA. The Biofactory Competence Center (BCC) is a not-for-profit organization based in Switzerland with the aim of providing training courses to the biopharmaceutical industry and related sectors, in order to supply a highly trained workforce. The BCC also provides technical services, including process design, development, and optimization.

Cell & Gene Therapy Insights 2022; 8(8), 827–834

DOI: 10.18609/cgti.2022.114

MICROBIAL FERMENTATION FOR PDNA

Viral vector manufacturing requires considerable amounts of pDNA. With the rise of mRNA technology over the last 2 years, the need for pDNA has further increased. Manufacturing pDNA utilizes *E. coli* due to its high growth rate and well characterized nature. However, to achieve high cell density fermentation with *E. coli* cells, a fed-batch process is required.

Supercoiled DNA is much more efficiently transcribed than relaxed DNA, as supercoiling serves a regulatory purpose and increases the rate at which proteins locate binding sites on the genome. High quality pDNA (or pDNA with a high supercoiled ratio) requires a highly monitored and controlled process with an induction phase. To optimize critical timepoints such as induction and harvest, in-line monitoring tools delivering real-time data are important, especially at high growth-rates. Research has shown the optimal induction timepoint to be at an optical density (OD) of ~35 to increase the ratio of supercoiled pDNA.

OD at 600 nm is used to follow the *E. coli* growth in real-time and to identify optimal induction and harvest time points. The current monitoring and control of *E. coli* processes is mainly performed using off-line measurements, such as ultra-violet visible (uv-vis) spectroscopy. Standard uv-vis spectrophotometer measurements require sample dilution and background interferences of media components must be subtracted from these measurements. Current in-line measurements use photometric sensors, such as Mettler Toledo Lasentec or Hamilton, though these show limitations at high cell densities >40 OD.

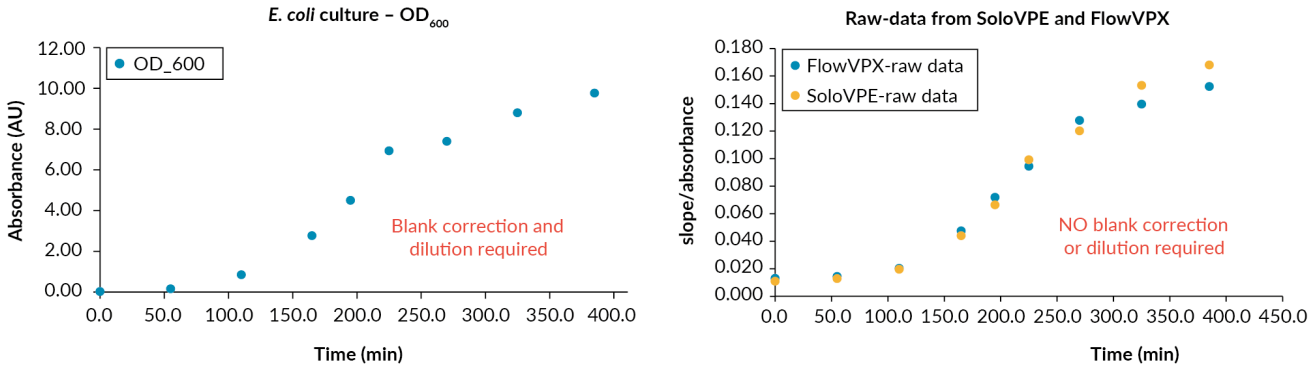
CTECH™ SOLOVPE® & FLOWVPX® SYSTEMS VERSUS STANDARD UV-VIS SPECTROPHOTOMETER

In this experiment, variable pathlength technology (VPT) is used to monitor and control *E. coli* fermentation. VPT follows the Beer-Lambert law, where absorbance is proportional to solute concentration. This allows pathlength variation according to concentration to ensure absorbance stays within the linear range.

The benchtop SoloVPE System consists of a normal cuvette, a detector, and a Fibrette for extension of the light source to adapt the pathlength. The FlowVPX System is an in-line measurement device containing a flowthrough chamber, detector, and a Fibrette to adapt pathlengths. These two technologies were applied in order to monitor and control fermentation with *E. coli* cells.

FIGURE 1

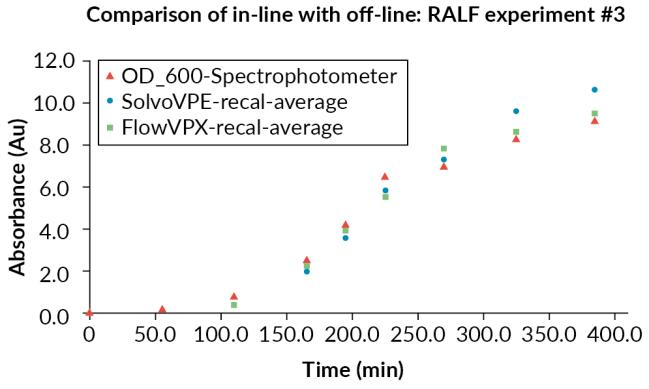
***E. coli* culture OD600 measured with standard spectrophotometer (left). Slope-absorbance raw data measured with SoloVPE System and FlowVPX System (right).**

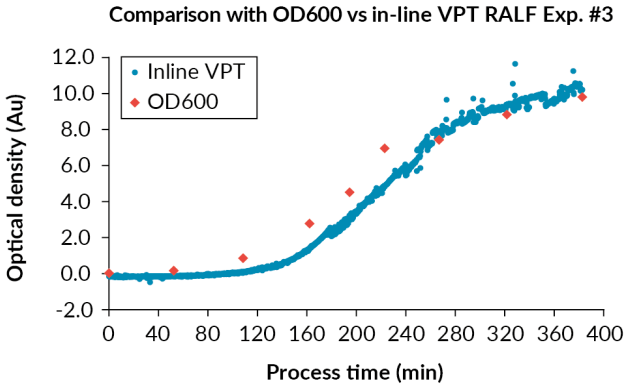


DOI: 10.18609/cgti.2022.114

FIGURE 2

Comparison of in-line and off-line measurements with a standard spectrophotometer and variable pathlength spectroscopy for each sampling time point (left). In-line measurement in real-time with the FlowVPX System and the off-line measurements with the standard spectrophotometer for the sampling time points (right).





A 3.5 L fermenter was filled with 2.0 L YPG (5 g/L of glucose) and autoclaved. For the *E. coli* inoculum, wild-type K12 was cultivated overnight in a 250 mL shake flask. Standard fermentation parameters of 37°C, pH 7.0, and pO2 > 35% were used. Agitation began at 400rpm and was increased throughout the culture to 800 rpm to maintain pO2. The fermenter was inoculated at a starting OD of 0.05 and off-line samples were taken every 30 minutes to measure OD with both the spectrophotometer and SoloVPE System. A sample of fermentation broth was pumped continuously in a circle through the FlowVPX System using a dipping tube to measure the increase of cells in real-time.

The results are shown in Figure 1. Samples needed to be diluted and results being corrected with the blank for the standard spectrophotometer. No sample treatment was necessary for the SoloVPE System analysis or the online FlowVPX System analysis.

A linear correlation was found between the slope-absorbance and OD600 in the range of 0.02–11.0 OD with a R2 = 0.9804.

This fermentation was performed three times and for each fermentation run, the data of the SoloVPE and FlowVPX Systems were correlated with the OD data. The FlowVPX data showed good correlation with the OD600 measurements from 0 to 15%. These recalculations are shown in Figure 2.

The results demonstrate that *E. coli* cell growth curves can be monitored using at-line and in-line VPT systems with high accuracy and repeatability, without the need for dilution or baseline correction. The results show that the data is comparable to the standard spectrophotometer OD600 measurements. This study can be used as proof of concept for future experiments to determine the correct timing for the induction of pDNA production in *E. coli* cells. The ability to monitor the fermentation process in-line using process analytical technologies allows for the optimization of the process and possible yield increases for pDNA.

Future work will include implementation of the SoloVPE and FlowVPX Systems in a fed-batch process to reach higher cell concentrations. The FlowVPX System may be used as a feedback control to perform an induction step based on the cell concentration, which could also be implemented in the plasmid production process. The impact of certain media components will be monitored, including switching to a defined medium. There is also the possibility to adapt this method and implement these settings with other organisms, such as yeast, fungi, and algae.

Q&A with Tanja Buch & Ramsey Shanbaky

Q Will air bubbles impact your measurements at OD600?

TB: Yes, you must be careful to avoid air bubbles when you implement the dipping probe into the bioreactor. Any air bubbles in the continual feeding line which goes into the FlowVPX could cause interference. We saw this when we had higher agitation and higher aeriation levels, as we had some interferences in the FlowVPX System data. Therefore, it is important that the dipping probe is implemented in a u-shaped form, so that the inlet is pointing upwards in the bioreactor.

Q Can the in-line system measure metabolites?

TB: It cannot directly measure the different metabolites such as sugar levels or other byproducts. This is why we have no expected interference. However, you can measure using standard absorbances – for example, NADH level. This occurs within the cells so should not interfere with measurements.

Q Is the flow cell autoclavable?

RS: Yes, the cells are autoclavable. In this study, Tanja used the 3 mm size, but we also have 10 mm and 22 mm sizes available. The materials used in the flow cell are stainless steel 316 L, quartz, and EPDM. They are all able to withstand the temperature of an autoclave.

DOI: 10.18609/cgti.2022.114

Q Why is buffer correction not required?

TB: One advantage of VPT devices is that they can measure up to ten data points. In this case, they measure the same sample at 10 different pathlengths. They measure the absorbance, and the system automatically makes a linear regression of these 10 datapoints, which provides you with the slope.

If you were to have some background interference – for example, due to the color of the medium – you would see a shift in the linear regression in the slope. However, the slope itself should be the same. Therefore, there is no interference from the medium nor buffer correction required.

Q Can you automate the process?

RS: If you have standard systems that run software, like DeltaV, the FlowVPX System has a way to connect through OPC Unified Architecture (UA), the standard communication protocol that allows the sending of signals back and forth between software packages.

TB: We have put some work into automation. We started a cooperation with bio-engineering to get access to their control systems. In the future, we wish to experiment with a feedback control loop from the data we obtain with the FlowVPX System, in order to start the pump, for example, or to increase the temperature to start the induction and manufacturing of the pDNA. Automation is definitely feasible, depending on the bioreactor systems you have.

Q How easy is it to sterilize the SoloVPE and FlowVPX Systems? Are there any concerns with contamination?

TB: It is easy to sterilize. To do so, you connect the entire loop in which you are pumping the system. In this case, the benchtop reactors will then need to be autoclaved. You can remove the flow chamber from the FlowVPX System device, but we can also put everything together in the autoclave, so everything comes out sterile.

Q What volume of culture is in the feeding line at any one time, and how quick is the cycle time?

TB: We used thin (3 mm diameter) tubing and tried to keep the loop as short as possible, at 2 mm long. We pumped it at a rate of 40 mL/min to avoid any sedimentation.

The cycle time of the FlowVPX System is around 30 seconds, giving you one to two measurements per minute.

BIOGRAPHIES

TANJA BUCH is a Biosystem Engineer with a PhD in Bioprocess Engineering. She has over 10 years of experience in the cultivation of mammalian cells and microorganism as well as the purification of recombinant proteins using filtration and chromatographic tools. Tanja has worked in several cooperation's with large biopharmaceutical companies to support them with process development, process optimization or analytical method development for their processes. In 2015 she took part in the design and set-up of the Biofactory Competence Center (BCC) in Fribourg, Switzerland, a training and research center for the biopharmaceutical industry. Within the BCC, Tanja is responsible to deliver training courses to the biopharma companies and to design and perform research projects for the industrial partners in all areas of bioprocess manufacturing.

RAMSEY SHANBAKY spent the first 8 years of his career working in R&D, Manufacturing and Product Management designing and building fiber optic products for high powered laser delivery in the medical device industry. In the first part of his tenure at Repligen, he worked alongside his customers to implement the CTech™ SoloVPE® System within biopharmaceutical accounts. For the last 5 years, he has been partnering with customers to implement the CTech™ FlowVPE® and FlowVPX™ Systems for in-line concentration measurements both in the process development and GMP manufacturing areas for bioprocessing. He holds an BSEE from Purdue.

AFFILIATIONS

Tanja Buch
Technical Director, Biofactory Competence Center (BCC)

Ramsey Shanbaky
Director, In-line Applications, Bioprocess Analytics, Repligen Corporation

IN MEMORY

Ramsey Shanbaky

(August 2, 1978 – July 16, 2022)

Ramsey Shanbaky was an extraordinary member of our Repligen family. His larger-than-life personality manifest itself through his passion for living and an equally large heart. Technically, Ramsey was comfortable speaking about laser systems, medical devices, bioprocessing workflows and of course Slope Spectroscopy, but in many ways his personality was his superpower. Curious, creative and truly genuine to his core, Ramsey was able to forge strong collaborative relationships with his colleagues, customers and anyone and everyone who had the good fortune to meet and work with him.

Ramsey's passions certainly did not begin and end with his professional endeavors. The greatest priority in his life was his family and he would light up when talking about kite surfing with his wife or playing Minecraft or snowboarding with his son. His engineering and analytical talents were not directed solely toward spectroscopic endeavors, his home brewed coffee roasting system and process control techniques produced coffee roasts that were fought over whenever those precious vacuum sealed bags made their way into the office.

Ramsey's enthusiasm, his energy, his ideas and his friendship are dearly missed.

He was a bright light, gone too soon.

DOI: 10.18609/cgti.2022.114

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: *Ramsey Shanbaky is a Repligen employee and stockholder.*

Funding declaration: *Repligen provided the equipment (SoloVPE and FlowVPX) as well as the consumables for the SoloVPE (cuvettes and fribrettes).*

ARTICLE & COPYRIGHT INFORMATION

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

Webinar recorded: *Jun 1 2022;* ***Publication date:*** *Sep 5 2022.*

Better insight.
Better control.
Better process.

In-line concentration monitoring for better process control

The more you know about your process as it's happening, the better you can scale, and the better you can optimize and predict the behaviors you need from your process.

The CTech™ FlowVPX® System accurately measures concentration in-line, providing you with real-time concentration data and giving you process insights that, up until now, were unavailable. With the FlowVPX System, you are able to increase your understanding of critical process parameters and make split-second decisions for enhanced biologic product consistency and quality.

Upgrade your process control with the FlowVPX System.

Take Control

REPLIGEN

FASTFACTS

Alleviate crucial challenges in scaling up gene therapy manufacturing

Emily Moran, Center for Breakthrough Medicines

By 2025, the FDA expects to receive upwards of 200 investigational new drug (IND) applications for cell and gene therapies annually, with an estimated approval of 10 to 20 therapies each year. This unprecedented growth comes with unique challenges for advanced therapy developers and manufacturers, which often center around three core must-haves: quality, speed, and flexibility.

Cell & Gene Therapy Insights 2022; 8(8), 1135; DOI: 10.18609/cgti.2022.167

With biotech having differing needs when ramping up AAV production, designing a purpose-built facility **(Figure 1)** is paramount to enable a seamless delivery process. Key factors to consider while planning for scalable gene therapy manufacturing include:

- **Diversification:** variety of equipment to de-risk the supply chain and process
- **Materials:** optionality with consumables & equipment
- **Optimization:** creation of process characterization opportunities
- **Early comparability studies:** pilot process development labs provide valuable insight into equipment comparability
- **Multi-platform consideration:** early investment into process definition on multiple platforms to provide agility in the future
- **Regulatory readiness:** commercial-ready suites and FDA-reviewed facility design

Concentrating end-to-end capabilities for production, harvest, purification, formulation/fill, in-process analytics, cutting-edge equipment for high-throughput characterization, and productivity in a single site can reduce the production bottleneck.

A scalable platform process approach adapted to client needs addresses the current capacity crunch.

In the case study **(Figure 2)**, CBM was able to successfully complete a client's tech transfer and pilot run first-time right in under 4 months. The client had an immediate need for pre-clinical material generation at the 200 L production scale for an AAV, purified to drug substance.

Challenges existed with the supply chain regarding equipment and material procurement (single-use materials and equipment such as bioreactors). Covid-related backlogs and delays threatened on-time completion of the run. While the client owned development facilities, they did not have the personnel to complete process development and the first run. Furthermore, the client process was still under development and so the process definition was evolving throughout the transfer process.

To begin, expectations and key risks were aligned between the client and CBM to ensure resources were efficiently deployed and avoid surprises. A highly collaborative structure was implemented to:

- Connect technical SMEs between parties to effectively transfer the process and quickly resolve issues
- Identify gaps in equipment and materials to work through novel solutions
- Manage and frequently review detailed schedules and risk registers

CBM began by understanding the client's goals, then collaboratively outlining the transfer process, key stakeholders, and likely risks, to ultimately formulate a flexible, client-specific way of working and service to meet the client's objective.

Safety and regulatory guidelines serve as the foundation for design. Additionally, mapping out the procedural controls must be considered to ensure optimized personnel, equipment, and waste flow. Upfront investment into engineering controls for segregation will pay dividends in any multiproduct facility, whilst taking a strictly clinical approach may result in costly rework later.

Figure 1. Drivers that must be considered in design strategy

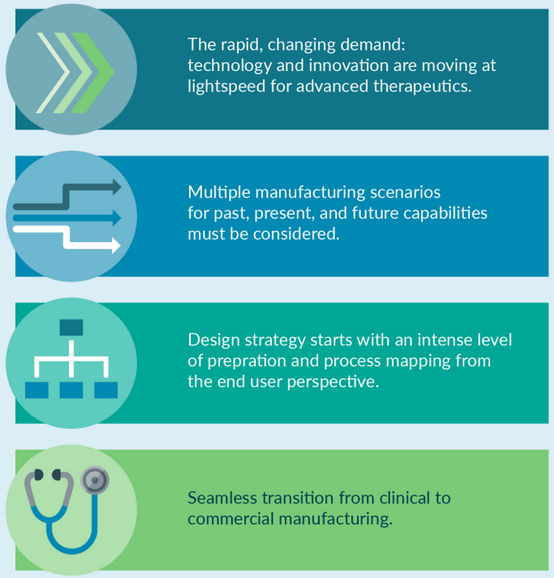


Figure 2. Case study: first-time right tech transfer and pilot run completed in under 4 months.

CELL & GENE THERAPY INSIGHTS

In partnership with:
CBM CENTER FOR BREAKTHROUGH MEDICINES

FASTFACTS

Digital data transformation: optimizing CGT processes and accelerating scale-up & tech transfer for manufacturing success

Teodor Leahu, Director of European Operations, IDBS

BRIDGING THE DIGITAL DATA GAP

Forward-looking companies are deploying cloud-based, 21 CFR Part 11 compliant, centralized data hubs as their persistent knowledge 'libraries' for process, product and patient data from R&D through clinical and commercial manufacturing and across the supply chain. These collaborative data platforms even capture relevant documents and notes and provide a full audit trail of changes to specifications and target control limits. This approach eliminates the risks associated with the traditional static 'transfer' of technology as the data remains persistent and available – even as staff, partners and facilities may change.

CREATE A DIGITAL DATA BACKBONE EARLY

Adopt a flexible, intuitive and validatable system that efficiently addresses growth needs regardless of the existing landscape. The first digital tool to move away from paper and Excel – and a springboard or complement to other systems; such technology enables process development teams to precisely build out process steps and unit operations for each product in their pipeline, captured in a centralized knowledge base. Consequently, tech transfer is optimized across development, clinical trials, and commercial manufacturing while providing the PD team a process audit trail and clear view into previous rationale.

In order to support clinical production, drive tech transfer and accelerate commercialization, a process information management platform such as Skyland PIMS must be able to achieve the following:

• Simplify data acquisition & management • Enhance ›process monitoring & understanding • Elevate data visibility & integrity • Expedite batch release • Streamline business & regulatory reporting • Improve operational efficiency • Reduce cost of compliance

Go to market challenges for cell and gene therapy.

Cell type variability

Cell and gene therapies contend with variable input material from apheresis and other sources of human cells, underscoring the need for process understanding and rigorous target control limits to ensure a consistent output.

Manufacturing process control

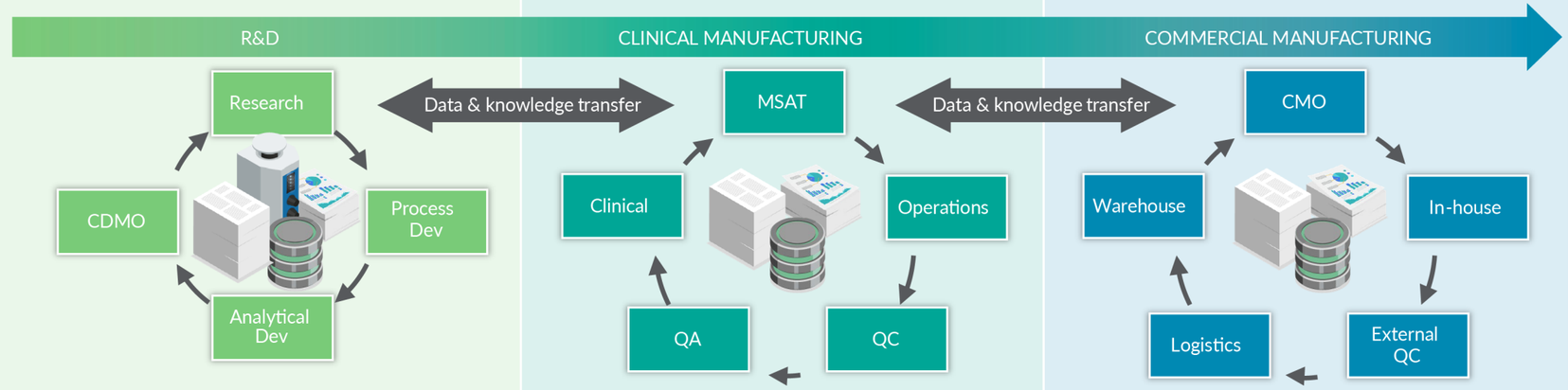
The overarching challenge that manufacturers face is establishing a closely choreographed supply chain. This requires compliant collaboration platforms across teams, sites and partners to establish process visibility.

Cost optimization

Without the economy of scale offered by traditional medicine, the novelty of autologous therapies carries high costs. For many use cases, this is a non-starter with patients and payers without profoundly reducing material and manufacturing costs.

STAKEHOLDER COLLABORATION

Process information management platforms drive collaboration with cell and gene therapy suppliers, apheresis collection points, and clinical trial sites further enhancing operational efficiency and oversight. This enables clinical outcomes to be linked with product critical quality attributes to pinpoint the positive responses seen in patients. What's more, development and manufacturing teams gain the resources needed to concisely present complex autologous and allogeneic treatment processes and improve buy-in from stakeholders – clinical staff, executives, board members, capital providers, payers – via the software's process definitions and analytics.



- Reduce data entry, verification and calculation time and errors
- Track protocol changes
- Rapid comparison of experimental results
- Flexibility to attach protocols/reports/notes and link to URLs

- Easily establish and monitor CPPs
- Continuous process verification (CPV) planning
- Multi-site batch monitoring
- Batch Genealogy - raw materials, QC samples
- Increase RFT batches and reduce deviations
- Visibility of site to site process differences
- Validated platform with full audit trail
- Fast and easy mapping of batch related data

- Centralized data repository for batch related critical data e.g. process, QC, raw material
- CPV plans and APRs
- Data backbone connecting multiple data sources - MES, EBR, QMS, LIMS
- Coherence of data required by cross-functional groups - Manufacturing, QA, Regulatory, QC
- Report generation for senior management

KEY

Paper Spreadsheets Data systems Equipment

CELL & GENE THERAPY INSIGHTS

Cell & Gene Therapy Insights 2022; 8(8), 773; DOI: 10.18609/cgti.2022.116

In partnership with: IDBS

CELL & GENE THERAPY INSIGHTS

CELL & GENE THERAPY MANUFACTURING SCALE-UP/SCALE-OUT

SPOTLIGHT

INNOVATOR INSIGHT

Optimizing cell proliferation and function for immunotherapy with recombinant growth factors and cytokines

Martin Keough

As more and new immune cell therapies continue to be developed, the demand for Animal Component-Free (ACF) and GMP-quality recombinant human growth factors and cytokines has increased. Cytokine therapies have become extremely popular due to their effectiveness in the treatment of cancer and autoimmune conditions.
The use of GMP products is the gold standard for Cell & Gene therapy manufacturing. Especially the use of GMP recombinant human interleukins as companies prepare for clinical trials or scale-up manufacturing of their immune cell therapies. Interleukins (ILs) are a group of cytokines that regulate immune and inflammatory responses. Interleukins also regulate cell growth, differentiation, and motility. The discovery of Interleukin (IL-2) as a "T-cell growth factor" (TCGF) in 1976 revolutionized the fields of basic immunology research and immunotherapy for human cancers.

Cell & Gene Therapy Insights 2022; 8(8), 1035–1043

DOI: 10.18609/cgti.2022.153

This article discusses CTGrade Recombinant Human Interleukins, which are designed to optimize cell expansion, activation, proliferation, and differentiation while providing consistent lot-to-lot biological activity and performance.

INTERLEUKINS: THE ROLE THEY PLAY IN THE REGULATION OF ADAPTIVE CELLULAR RESPONSES

Interleukins (ILs) are a group of cytokines that regulate immune and inflammatory responses, cell growth, differentiation, and

motility. The discovery of IL-2 as a T cell growth factor in 1976 revolutionized the field of immunology research and immunology for human cancers and set the stage for the use of interleukins, along with a host of cytokines and growth factors for therapeutic development. Cytokine therapies have since become extremely popular due to their effectiveness in the treatment of cancer in autoimmune conditions. As new immune cell therapies continue to be developed, the demand for animal component-free, beta-lactam free, and cGMP quality recombinant human growth factors and cytokines is increasing.

CTGrade recombinant proteins are designed to optimize the expansion, activation, proliferation, and differentiation of T cells, natural killer (NK) cells, B cells, and chimeric antigen receptor (CAR) T cells. The proteins support basic, translational, and clinical research as well as commercial applications. Additionally, CTGrade proteins are formulated to reduce variability and ensure predictable workflow performance.

Implementing the use of cGMP proteins ensures consistent lot-to-lot biological activity and performance to support basic, translational and clinical research as well as commercial applications.

ACHIEVING OPTIMAL RESULTS THROUGH SCIENTIFICALLY DRIVEN SELECTION OF PRODUCTS

An experiment was set up to measure the performance between peripheral blood mononuclear cells (PBMC) expansion of CTGrade rhIL-2 lots from Shenandoah Biotechnology Inc. Three different concentrations of CTGrade recombinant human IL-2 (100 IU/mL, 200 IU/mL, and 1000 IU/mL) were tested across three donors, using a non-GMP control.

IL-2 driven growth trends were similar across all three donors (Figure 1). Donor-to-donor variability was observed but the overall performance between PBMC expansion of the cGMP lots versus the control lot was insignificant. The lower IL-2 concentrations consistently expanded the least, while the higher concentration lots of CTGrade IL-2 showed continuous expansion over the 13 days.

Cell viability was also assessed using the same culture conditions (Figure 2).

Across all three donors, 80–90% viability was maintained in the presence of IL-2. The drop in cell viability on day three was an experimental issue driven by the magnetic antibody beads being read as dead cells during expansion. The effect was reduced in subsequent days.

Cell diameter was measured to further assess the IL-2-driven expansion (data not shown). Overall variability was seen across donors as expected, but in all cases, the diameter was maintained.

T cell expansion data presented was generated using human PBMCs and expanded in the presence of 200 IU/mL CTGrade recombinant human IL-2. Activation markers and exhaustion markers were then measured. As shown in Figure 3, activation and exhaustion markers reflected healthy cell growth kinetics over two weeks of culture in 24-wellplates. CD69, an early activation marker, peaked at approximately the same time as the exhaustion marker, PD-1. CD25, a late-stage activation marker, peaked at the end of the first week of culture and dropped to baseline by day 13.

Cytotoxic and immunomodulatory support were also measured. Figure 4 shows that the use of PRIME-XV T cell chemically defined media (CDM) supported the cytotoxic and immunomodulatory function of expanded T cells. Intracellular cytokine staining for interferon-gamma, tumor necrosis factor alpha, and interleukin 4 (IL-4) were measured in the various populations as well as the CD107a, granzyme B, and perforin for CD8 T cells.

These data show that cells expanded in the presence of CTGrade IL-2 and PRIME-XV media can express proteins and markers

DOI: 10.18609/cgti.2022.153

FIGURE 1

PBMC cell counts throughout the course of a 13-day culture.

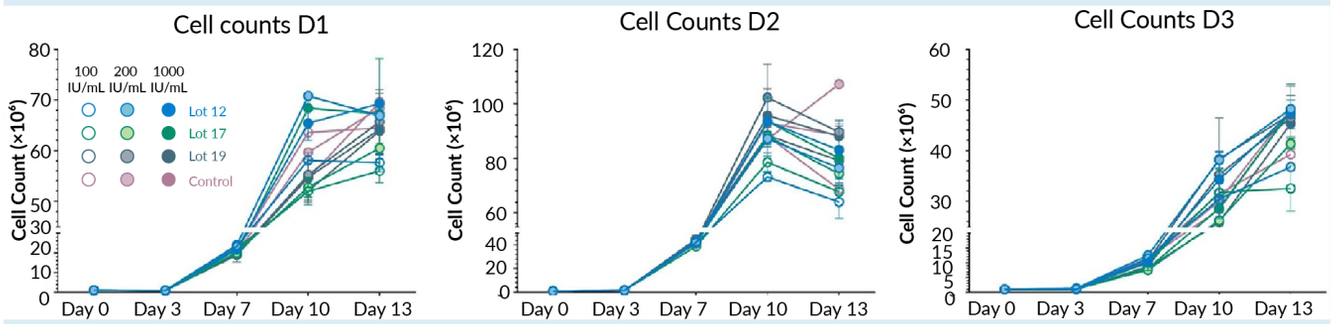


FIGURE 2

PBMC viability across a 13-day culture..

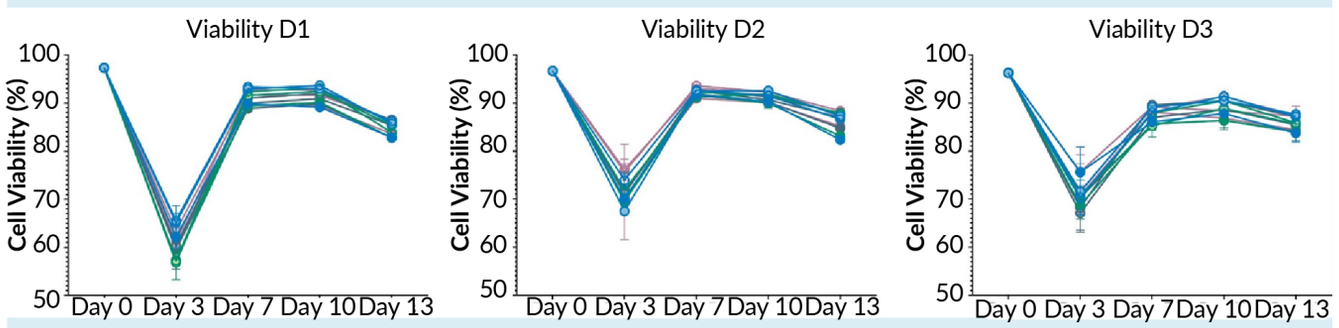


FIGURE 3

Dot plot flow cytometric analysis was taken on day 3 and day 13 using CD62L, CD25, CD69, and PD-1, and a representative blot.

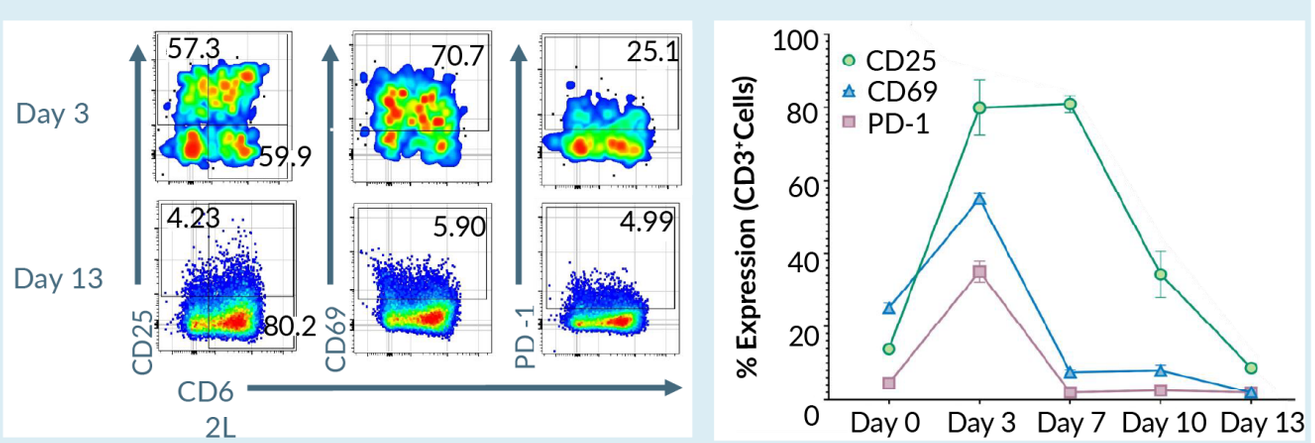
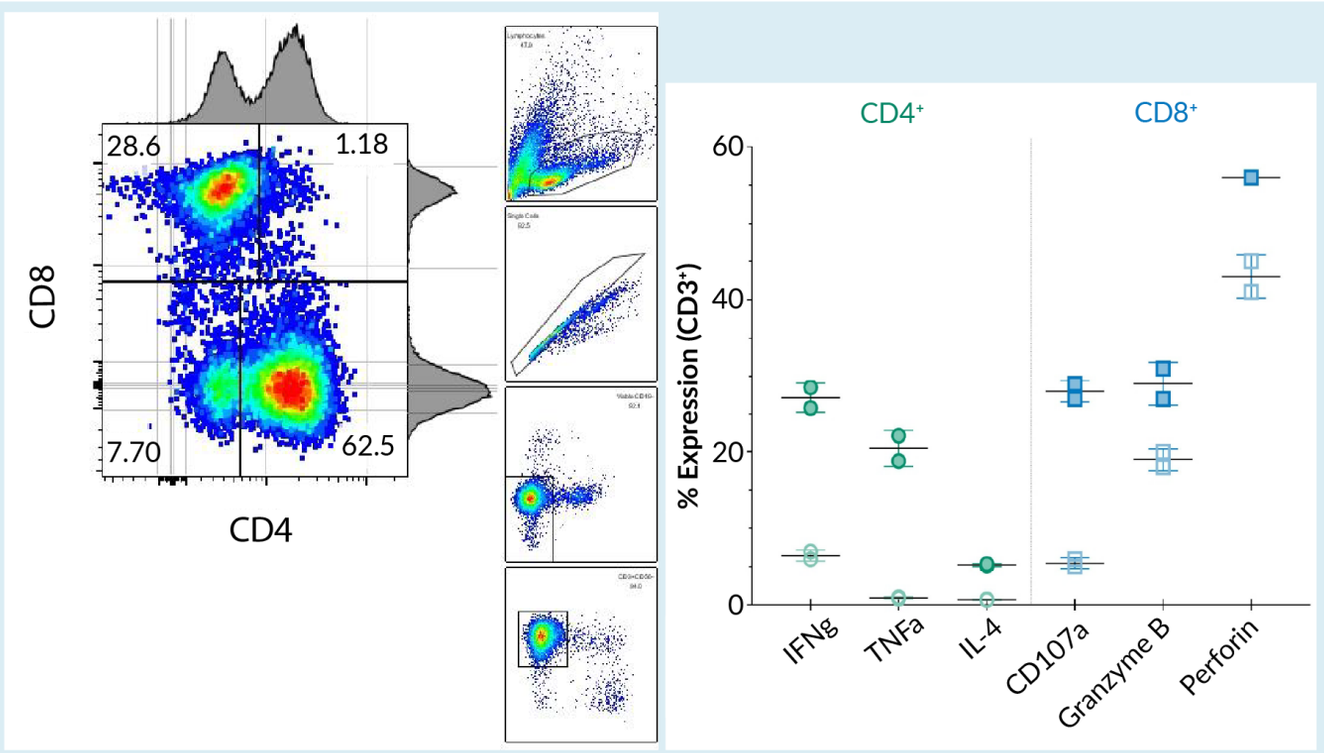


FIGURE 4

Dot plot and flow cytometric schematic for cytotoxic and immunomodulatory support analysis of PBMCs with the addition of PRIME-XV T cell CDM.



indicative of activation, suggesting good support and expansion with these two products.

THE IMPORTANCE OF PARTNERSHIP-FOCUSED SUPPLIERS

When designing cell or gene therapy processes, making the right decision the first time about raw materials is critical to ensure a predictable workflow. From the cell culture media to the development process, the selection of a partner who can deliver predictable workflow performance is key. To achieve the desired quality, yield, and function, the process should utilize the highest quality raw materials that meet rigorous quality and regulatory expectations. Raw material suppliers utilize critical controls to ensure the rigorous evaluation of the components used to develop recombinant proteins. The components used to make cell or tissue-based products can vary due to their complexity, thus having a significant impact on the final product quality assurance. Media manufacturers must clearly understand the complexity and therefore produce materials that are safe to use in cell therapy applications.

The customer and supplier partnership is the focus of FUJIFILM Irvine Scientific and Shenandoah Biotechnology. This means providing the customer with the highest quality products, and supporting them in their regulatory agency filings, implementing quality agreement audits using cell processing centers, and applications for manufacturing support. They aim to support approval for advanced therapy medicinal products through quality regulatory support and expertise, provision of technical information, and implementation of drug master files for use by the end user.

DOI: 10.18609/cgti.2022.153

Q&A

Roisin McGuigan, Editor, BioInsights
speaks to (pictured) **Martin Keough, Chief Executive Officer, Shenandoah Biotechnology, a FUJIFILM Irvine Scientific company**

Q What types of variability can be observed due to differences in the quality of an interleukin?

MK: Throughout the years, Shenandoah Biotechnology has sought to reduce inter-lot variability, which can vary dramatically across the different suppliers that are available.

These differences are usually observed in the process and can be driven by the source of the material. Shenandoah produces several products in E. coli, but we know that varying strains can have a dramatic impact.

Shenandoah is reducing that variability between lots by instituting testing methods for international units and increasing the documentation and quality around each of the products. We analyze each lot with strict standards for quality control and release. By adhering to those standards, we reduce the variability between the manufacture of each lot of ILs.

Q What are the key elements of a quality system that I should look for from a provider of recombinant ILs?

MK: The end user should look for a manufacturer that adheres to recognized guidelines (e.g., good manufacturing process, [GMP]) and standards set by igroups such as International Organization for Standardization. They should adhere to the Code of Federal Regulations (CFRs) in the US, or the appropriate similar regulatory agencies in Europe and across the world.

You should look for testing within the quality system to recognize guidelines. There are a number of guidelines that are harmonizing, including the United States Pharmacopeia (USP), the International Council on Harmonization, the European Pharmacopeia, and the Japanese Pharmacopeia standards.

Shenandoah has excelled at both the quality of product support we offer, and our transparency in the manufacture of the products. Many of us working here have been on the other side of the bench in needing information about the quality of the products. We understand what it is like when you are interacting with companies, and it can be difficult to get the

answers you need. As we appreciate those struggles, we seek to provide the highest quality products with the greatest amount of transparency, to help support the end user in their needs for our products.

Q What is the difference between the research use-only (RUO) and GMP GF media that you offer?

MK: One of the things we have strived for in our collaboration is to provide as little differentiation between RUO and GMP as possible. During the manufacturing process of the recombinant growth factors, we manufacture all our products off the same batch records to the same standards as if the product was going to be released as GMP.

There are two reasons for this. We do not have to segregate and run the risk of having cross-over and problems. The highest differentiation occurs is at the backend. There is a lot more testing that goes into the GMP product to ensure its release. Similar testing is done on the RUO, but not as many tests are run.

As we manufacture under the same guidelines, batch records, and processes, our end user receives a smooth transition from preclinical studies to clinical studies. That means they can be assured that an RUO product is going to be of the same quality and performance when they transition to the GMP version of that product.

Q I've seen suppliers indicate that they have a specific area of their cytokine production facility that is beta-lactam-free. What should I look for in terms of this when selecting a source?

MK: The CFR and US food and drug administration guidelines are driven mainly by manufacturers producing antibiotics, who suggest that beta-lactam antibiotics and non-beta-lactam antibiotics should be produced in two separate facilities, with separate support systems to prevent any cross-contamination.

As we progress forward and standards begin to change, customers must look for true beta-lactam-free production, where there is no beta-lactam whatsoever used during the manufacturing within a particular building or facility for that product. Although we can do testing for levels of residual beta-lactam, we cannot ensure that sensitivity to residual beta-lactams will not exist in a patient given the cell therapy.

Q What are the advantages of using GMP IL-2 from Shenandoah, versus other commercially available products on the market?

MK: Shenandoah Biotech has strived to meet, exceed, and begin to define the industry standard for the use of recombinant growth factors and cytokines. IL-2 is our first offering and given the importance of this recombinant growth factor to the emerging field of CAR T cell therapies, having a reliable source for IL-2 is critical.

DOI: 10.18609/cgti.2022.153

We offer two versions of IL-2. Many are familiar with IL-2s from the wild-type, while others use the clinically marketed IL-2 therapy known as aldesleukin. Because we have both offerings, it allows the transition of a group using either the wild-type or the aldesleukin sequence directly. Shenandoah manufactures to strict standards for endotoxin levels, purity, activity, and standardization of all IL-2 lots to the international reference standard. The product offerings allow the transition directly to the IL-2 used by the end user, as well as the direct comparison based on activity for a seamless transition of our product into their workflow.

Q Beyond IL-2, what ILs are needed to produce cell-based therapies?

MK: The list is ever-expanding. A great deal of research has been going on, and right now, IL-7, IL-15, and IL-21 are the ones that we are receiving the most inquiries about.

Staying in the immunological space, CT Grade releases are coming for IL-3, IL-6, IL-10, IL-4, nCSF, GM-CSF, and others. Transitioning over to stem cell support, we offer FGF2, EGF, PDGF-BB, and other growth factors to help support the maintenance of stem cells as well as their differentiation.

With the extensive catalog of recombinant growth factors and cytokines that Shenandoah provides, as well as the chemically defined media produced by FUJIFILM Irvine Scientific, we serve as a one-stop shop for the clinical development of advanced cell therapies by the end user.

Q Which cytokines do you use to differentiate or expand T or NK cells?

MK: Presently, most of our work for T and NK cells uses IL-2, IL-7, IL-15, and IL-21. We are using this limited set as we begin to mature the CTGrade offerings and vet the cytokines in chemically defined media offerings from the FUJIFILM Irvine Scientific Shenandoah collaboration.

We continue to vet the literature and speak with key stakeholders in this arena surrounding the phenotypic properties desired. Although we have a core group of cytokines, that list is changing and expanding for particular use. We have ever-expanding data in cell culture media and can work with end users to tailor specific cytokines for their ultimate needs.

Q If it is needed, can you customize GMP lots to be more specific to customer applications?

MK: Yes, customization is possible. This is something that we are presently involved in and expanding. We can manufacture lots specific for the end user, providing them with consistency from run to run, and qualifying lots for subsequent use in manufacturing.

We seek input from the end user for their desired needs, and we routinely have calls with groups that need this ability and capability. Shenandoah Biotechnology is poised to support

customization of growth factors in the medium, from a high quality and customer support perspective.

Q What additional cytokines will you be providing in the future?

MK: I will not go through all 300, but as mentioned before, we offer IL-2, -7, -15, 21, -3, -4, -6, -10, basic FGF, PDGF-BB, VGF, GM, CSF, MCSF, BDNF, GDNF, NT3, NT4, NRG, and Beta1. This list is driven predominantly by our first offerings from inquiries from current customers as well as new inquiries from prospective customers.

The catalog is extensive, and we have an appreciation of what end users' needs may be, so we are well positioned to transition any of our growth factors into the GMP space, when needed, based on any customer inquiry. We have had inquiries ranging from advanced cell therapies to the artificial meat space, and into other unique aspects and applications that require growth factors. Our flexibility in transitioning those growth factors into GMP is another process advancement in adherence to the quality and support of the advanced cell therapies and new technologies that FUJIFILM Irvine Scientific and Shenandoah seek to support.

AFFILIATION

Martin Keough, PhD
Chief Executive Officer,
Shenandoah Biotechnology,
a FUJIFILM Irvine Scientific company

DOI: 10.18609/cgti.2022.153

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 FUJIFILM Irvine Scientific company. The author has no other conflicts of interest to disclose.*

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 based on an Webinar with Martin Keough carried out on Aug 3 2022.*

Webinar held: *Aug 3 2022*; ***Revised manuscript received:*** *Aug 31 2022*; ***Publication date:*** *Sep 29 2022*

CELL & GENE THERAPY INSIGHTS

CELL & GENE THERAPY MANUFACTURING
SCALE-UP/SCALE-OUT

SPOTLIGHT

INTERVIEW

Making the switch from autologous to allogeneic cell therapy

Elena Matsa & Andy Holt

ELENA MATSA has expertise and experience in a broad array of functions essential to successful allogeneic cell therapy manufacturing, including iPSC differentiation, genetic manipulation, phenotypic assay development, efficacy and safety assessment of therapeutic modalities, and in vitro disease modeling. For Cellistic and its affiliate company Ncardia, Elena manages key strategic technical projects, provides scientific leadership, and contributes to project proposals. After earning her PhD in stem cell biology in 2010, she worked as a post-doctoral research fellow at Stanford University, studying iPSC technology. She transitioned to the biopharmaceutical industry in 2017, working as a Scientist and, later, Director for drug discovery research. Elena's work has been published in *Cell Stem Cell*, *Nature Medicine*, *Science Translational Medicine* and *European Heart Journal*, among others.

ANDY HOLT brings more than 15 years of experience in cell and gene therapy to Cellistic. In his prior roles, he held business development, corporate development and management positions for CDMOs, driving commercial strategy and growth in adeno-associated virus (AAV) gene therapy manufacturing. In his role at Cellistic, Andy leverages his experience in scaling up cell and gene therapy platforms to help Cellistic clients reach their goals in allogeneic cell therapy.

Cell & Gene Therapy Insights 2022; 8(8), 935–943

DOI: 10.18609/cgti.2022.139

Q Could both tell me a bit about your backgrounds, and the current work you do?

EM: My story starts with a PhD in stem cell biology back in 2006, when induced pluripotent stem cells (iPSCs) were first discovered. I focused on investigating the mechanisms of iPSC programming, which was really exciting at the time.

I continued with two post-doctoral fellowships, one at the University of Nottingham, and another at Stanford university in California. It was really nice spending some time in Silicon Valley, studying iPSC-based disease modeling.

After this, I transitioned to industry for a couple of years in South San Francisco, and then to Director of Drug Discovery Services at Ncardia. This journey was a very interesting one, and over 15 years I gained a lot of experience in iPSC programming, genome editing, assay development, and importantly differentiation of iPSCs to different lineages, such as heart cells, neurons, and immune cells. This prepared me for my current position at Cellistic where I lead strategic and scientific projects as Vice President of Cell Technologies.

AH: I have a bit of a different journey, and certainly less robust experience in the deep science that Elena brings to the table. My background has been on the commercial side of biotechnology for the last 15 years. I had a little scientific training – but especially in a place like Ncardia/Cellistic it is a teeny tiny bit of training!

I jumped over into cell culture formulation and media formulation work for a few years, then started working in services, which is something I really enjoy. I worked for a number of contract research and contract testing organizations. I was then lucky enough – and I can't claim anything other than luck was involved – to participate in some of the earlier projects in gene and cell therapy coming out of academia in the southeast United States where I am based.

From there I was exposed to how these things are made, and trying to figure out how to bring them into the clinic as novel therapeutics and novel modalities. I got associated with a couple of the larger CDMOs doing this work, and then most recently spent five years with a small gene therapy developer, AskBio, that was recently acquired by Bayer. As part of that acquisition we got to bring a lot of neat therapies forward and also spin out a couple of manufacturing companies.

When I came to Ncardia/Cellistic it was an incredible opportunity to be associated with a technology that's coming into its prime. I am very excited for the chance to be a part of this journey that so many people like Elena and others have worked for so long to bring forward.

Q What role do you predict iPSCs will play in the future of cell therapy?

> "With allogeneic cell therapies you can choose the donor to have healthy characteristics, and therefore have a better therapeutic outcome."
> - Elena Masta

AH: We are at a fascinating place for cell therapy right now. There is a common kind of vocabulary overlap – when we say cell therapy, we kind of mean immuno-oncology (I-O), but I think there are applications outside of that that iPSCs are especially powerful for. We have clear indications that the way these autologous cell therapies work is compelling and revolutionary.

However, there are severe limitations driven by autologous cell therapy as the choice for bringing these therapies forward, both in terms of access and in terms of manufacturing, that limit them reaching their full potential.

Obviously, I am biased, because I'm working for a company that has been fully devoted to iPSCs for a number of years. I strongly believe that iPSCs are the way forward to help these novel I-O drugs reach their full potential, reach the right number of people, and reach the right cost basis for everyone to have access.

My armchair quarterbacking here is that iPSCs will be the modality of choice to help allogeneic cell therapies truly come to the fore, dominate the space around I-O, and also get to some really interesting corners of the world where cell therapy can also reach. This could be tissue or organ regeneration; those next-generation applications of what you can do with a well-characterized, predictable source of human pluripotent cells.

EM: This is a great question, particularly for someone like me who has been in the iPSC field for over 15 years. To talk about the future of iPSCs in cell therapy is great, because this is something that we couldn't have imagined would be a reality not so long ago.

We've gone from using iPSCs in drug safety studies, in disease modeling, and understanding disease mechanisms and using them in drug discovery, to actually using them as a therapeutic.

This is now a very exciting reality. Immunotherapy and allogeneic iPSC-based cell therapy will quickly become dominant because of the benefits that they offer. For me, the future is in the application of iPSCs in the second branch of cell therapy, which is regenerative medicine. Being able to use these cells to create multi-cell-type artificial tissues for transplantation is where I see the future.

Q Can you expand on what you see as the key benefits of autologous versus allogeneic cell therapies?

EM: One of the big benefits is the time that it takes to create a therapy for a patient. In the case of autologous cell therapies, it takes at least six months to generate a

treatment for a specific patient. You have to isolate their cells in the hospital, bring them to the lab, expand them, modify them, and then put them back in the patient. This process is lengthy, and for patients that are suffering with severe disease it may be too long. Offering the option of an allogeneic cell therapy that is ready to use off the shelf at any time is a real benefit.

The other benefit is related to cost. Creating autologous cell therapies for every patient is very expensive – it can cost over half a million or even a million dollars per patient. Many families can't afford this. Having one production that can benefit multiple patients can reduce costs and therefore bring the therapies to more patients that need them.

Beyond the benefits to the patients, there are also benefits in terms of the manufacturing processes. If you are generating an allogeneic product that is the same for multiple patients, the process becomes more standardized, more predictable, and has a higher success rate. This also contributes to reduced cost.

Lastly, if you are interested in using a patient's own cells for their treatment, and the patient is carrying a mutation, the cells that you generate and want to transplant back into the patient for therapy will carry the same mutation. This may limit the function of the transplanted cells. With allogeneic cell therapies you can choose the donor to have healthy characteristics, and therefore have a better therapeutic outcome.

AH: From a scientific perspective Elena has definitely covered more things than I ever could. From the practical perspective of a patient, or someone running a manufacturing facility, there are also some exciting upsides to being able to move to allogeneic.

We have a suite of novel therapeutics with truly transformative results on the back of a cell therapy modality: autologous cell therapy. A few years ago, many liquid tumor cancers were incredibly challenging to treat in a meaningful way. All of a sudden, there are options that deliver 90+% complete response rates.

They rocketed through to approval, because they could save lives. It took decades of hard research to understand the human immune system in a deeper way, and to leverage the gene modification techniques needed to reach a therapeutic that is just incredible.

Where allogeneic can offer huge advantages is to allow a deeper understanding and a more complete answer to more patients than autologous can offer. There's that time sensitivity that Elena alluded to – these patients are dying. That is the reality. Being able to administer a meaningful therapeutic sooner, being able to allow them to continue on standard of care while that therapeutic is prepared, and also being given the luxury of time to fully characterize and fully release these large batches of cells that don't have the variability that autologous therapeutics do, helps to de-risk an exciting and dynamic therapy even further.

From the patient perspective that is exciting. From the manufacturing perspective, as someone who has spent a lot of time thinking about manufacturing strategy and those sorts of things, we can move the manufacture of these cell therapies into a much more traditional biomanufacturing workflow. That allows for many more doses and a much cheaper footprint and operational envelope to work within.

It also eases up the regulatory burden a little bit, because we have plenty of time and plenty of resource to answer questions the regulators may pose to us.

DOI: 10.18609/cgti.2022.139

Q What do you see as the biggest obstacles to successful iPSC-based allogeneic cell therapy development – and how can they be addressed?

EM: Nothing comes without downsides, does it? For allogenic iPSC-based cell therapies, the main obstacle – and I think what the regulatory authorities are most concerned about – is safety. For iPSC-based products the safety concern is primarily their karyotype stability. As the cells are cultured in an artificial environment, they have sensitivity to gaining karyotype abnormalities that give them an advantage for growth in cell culture. The programming and genome editing are processes that these cells are sensitive to, and if they are stressed during these processes, they give themselves a survival advantage by gaining karyotype abnormalities.

This can be overcome by thorough genotypic screening, and there are very good technologies to achieve this. There is the traditional G-banding, and also slip karyotype analysis. A thorough characterization of master cell banks and final products can help overcome this obstacle quite easily.

The second concern is the potential for carcinogenicity. Since these cells have the ability to differentiate into any cell type in the body, they could also differentiate randomly if they are not in the right environment, leading to tumors. This is a small risk in our view, and there would need to be a large population of residual stem cells in the end product to have an increased risk for carcinogenicity. Again, assessment and selection against the non-desired cells in the final product can easily help overcome this obstacle as well.

Then there is the obstacle of histocompatibility. Because you are transplanting cells into a patient that are not their own cells, you need to make sure that the HLA molecules are compatible. Otherwise this can lead to the host cells killing the implanted cells before they have had a chance to have a therapeutic effect. Alternatively, the graft itself can kill unwanted host cells. Typically with technologies such as genome editing or creating HLA homozygous donor banks this can also be overcome.

Another obstacle is the manufacturability, and this is something that we focus on a lot, considering our expertise. Because iPSC-based allogeneic cell therapies are fairly new, there is a lack of equipment, media, matrices and so on, in order to achieve GMP-compliant scaled manufacturing.

This is something that we work on continuously. It is important to start this type of thinking early and communicate with developers of devices and media in order to establish suitable processes, and together as a community help overcome these manufacturability obstacles so that we can bring treatments to the patients that need them.

> "...there is an exciting set of benefits waiting for anybody developing an allogeneic therapy, and really for all of us, if you are thinking about moving in that direction."
> - Andy Holt

AH: I don't have too much to add to Elena's answers here; that was as good an analysis as you could hear of the challenges. From a bit more of a layperson's perspective, a big challenge is just how new all of these issues are. In terms of allogeneic cell therapy clinical trials we are still in very early days. Both the developers and the regulators are figuring out together what is important, what the key risks are, and what can be characterized.

I am grateful that we are in a situation where there has been a flood of novel therapeutics coming forward over the last 10–15 years. This means there is a framework to work with the regulators in a really productive way on how to accomplish overcoming those challenges, and how to bring these things forward into patients.

One of the lessons that we've all learned is that all the research and development in the world doesn't have as much impact as reaching the clinic and reaching patients when it comes to understanding these complex and novel therapeutics. Seeing these living therapeutics in a human is the best way to really know what's going on. That comes with some risk, but this current state we are in is really conducive to bringing these forward. Fifteen years ago there were a lot more challenges in both educating the regulators, as well as collaborating with them on bringing these things forward and helping people.

Q What are the most important considerations for developing a successful cell therapy manufacturing process?

EM: The most important consideration is to know what the end goal is; what the target product is. That means defining the quality target product profile (QTPP). Understanding the qualities that you want to achieve from the beginning is the first step to having a successful manufacturing process.

Another consideration is to then build a process that is robust and reproducible. That means having a process that supports the same outcome for every batch of the product that is manufactured. It is not enough to do it once or twice; you need good reproducibility and you need to understand how a small change in one of the parameters of the process affects the end product. Having this complete understanding of the manufacturing process is very important.

The last consideration I will outline is traceability. This is what will help you get through audits and through IND filing. Nowadays this can be achieved with digitalization of the processes in order to achieve the traceability level that is needed for clinical use of the product.

AH: When I think about how to bring allogeneic cell therapy development forward, I focus more on the corporate strategy and the practical elements of how you want to deploy your capital early on. You can either design and build a facility on your own, or partner with a contract manufacturer.

Those are key decisions, and it comes down to a lot of the same questions Elena outlined. Do you view having a manufacturing asset and building up that team as really important? Do you have a robust manufacturing process already built? Are you ready for "prime time" with what you have, and you want to build infrastructure around that? Or do you need to partner

DOI: 10.18609/cgti.2022.139

because you have the immuno-oncology and cell biology expertise, but lack the quality, regulatory, manufacturing and scale up expertise that you need to reach your clinical trial?

If you are a therapeutic developer some of those fundamental questions have to be asked just as seriously, and at the same time, as you are considering your product profile. Otherwise you are going to be excited to start and then have nowhere to go, so to speak.

That then leads to a whole set of cascading questions about how you assess a partner, how you manage your budget, and all those sorts of factors, in order to be able to reach your end goal on time.

Q What is your key advice for making the change from autologous to allogeneic cell therapy development a practical reality?

AH: You need to consider what the end goal is from the start. Allogeneic cell therapy comes with some novel adventures. If you are developing an allogenic cell therapy right now, you are still an early adopter. The long-term benefits of a better cost basis for your therapeutic, hopefully easier broad reach to a number of patients, and better predictability and scalability, come with some short-term obstacles that Elena alluded to. We are all figuring them out together.

Obviously, we are pretty biased, and we would be excited about anybody thinking about that switch because we think that's the way the world is going, should go, will go – whatever set of verbs you want to use, we're in on it.

The key piece is knowing that you are going to get to break some new ground, and whoever you are working with is going to break some new ground with you, for a medium and long-term scenario that is really compelling and exciting. It will be a bit more of an adventure in the next 6–18 months, as opposed to an autologous development process. But there is an exciting set of benefits waiting for anybody developing an allogeneic therapy, and really for all of us, if you are thinking about moving in that direction.

EM: We have talked about the challenges of the process, and my key advice based on this would be to put the best team together, who can help expedite the product development process, and catch any common pitfalls. This could mean either building the team in-house or outsourcing it to an experienced partner. Either way, you want the best team to come together for the best outcome.

I would also advise retaining a focus on what the purpose of this is, which is to bring the best treatment to the most patients that need it. Doing this in an affordable way this could mean choosing allogeneic cell therapy.

Affiliations

Elena Matsa
VP of Cell Technology,
Cellistic™

Andy Holt
Chief Commercial Officer,
Cellistic™

ABOUT CELLISTIC

Launched in April 2022, Cellistic™ specializes in process development and manufacture of cell therapies based on human induced pluripotent stem cell (iPSC) technology. Its focus and expertise in iPSC reprogramming, differentiation, and expansion protocol development positions the business to be the partner of choice for innovative cell therapy developers to commercialize novel advanced therapies. Leveraging more than a decade of Ncardia's scientific and technical knowledge and experience, Cellistic™ possesses unique capabilities for the design and optimization of proprietary manufacturing platforms for iPSC-based cells that deliver quality products at scale. For more information, visit www.cellistic.com.

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: *Andy Holt and Elena Matsa hold leadership roles in Ncardia and have had expenses for travel and conferences paid by their employer, Ncardia. Elena Matsa participates as PhD Candidate supervisor in ITN ISNPIRE (#858070) and PersonalizeAF (#860974) multidisciplinary Marie Skłodowska-Curie Action grants, awarded to employer (Ncardia). She also lectures on 'Cell Therapy' at University College Cork (UCC); and has patents pending on HSC and T-cell differentiation from iPSCs.*

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 based on an interview with Elena Matsa and Andy Holt carried out on Aug 11 2022.*

Interview held on: *Aug 11 2022; Revised manuscript received: Aug 25 2022;* ***Publication date:*** *Sep 9 2022.*

DOI: 10.18609/cgti.2022.139

cellistic

Ascending cell therapy.

Not all big ideas are the same size.

Some are actually bigger.

Take allogeneic cell therapy. It has the potential to cure complex conditions not just for "the one," but also for "the many." But realizing that potential requires a manufacturing partner with foundational expertise in iPSCs and human biology. When you choose Cellistic, you'll get exactly what you need to scale your cell therapies efficiently, effectively and consistently: a track record of success in iPSC innovation and purpose-built differentiation and expansion platforms to help make your big idea a reality.

As big as you want to go, we'll go with you.

Get started at www.cellistic.com

CELL & GENE THERAPY INSIGHTS

CELL & GENE THERAPY MANUFACTURING SCALE-UP/SCALE-OUT

SPOTLIGHT

INNOVATOR INSIGHT

Accelerating cell therapy discovery & development with non-viral gene engineering

Xiaobai Patrinostro, David Hermanson & Scott Silaika

Gene engineering of immune cells is a powerful tool for creating advanced and novel cellular therapies. Currently, the critical step of cellular gene editing is primarily performed using virus-based gene delivery systems. Virus-based engineering methods are commonly plagued with long lead times, inconsistent batches, low cargo capacity, and high costs. Recently, advances in the non-viral transposon-based gene engineering have provided developers with an alternative gene engineering method that addresses virus-based platform limitations. Utilizing a 'cut-and paste' method of gene delivery, transposon-based systems are capable of stable genomic integration. This article discusses the transposon-based TcBuster™ platform, and its competitive advantages in cell and gene engineering.

Cell & Gene Therapy Insights 2022; 8(8), 1023–1032

DOI: 10.18609/cgti.2022.152

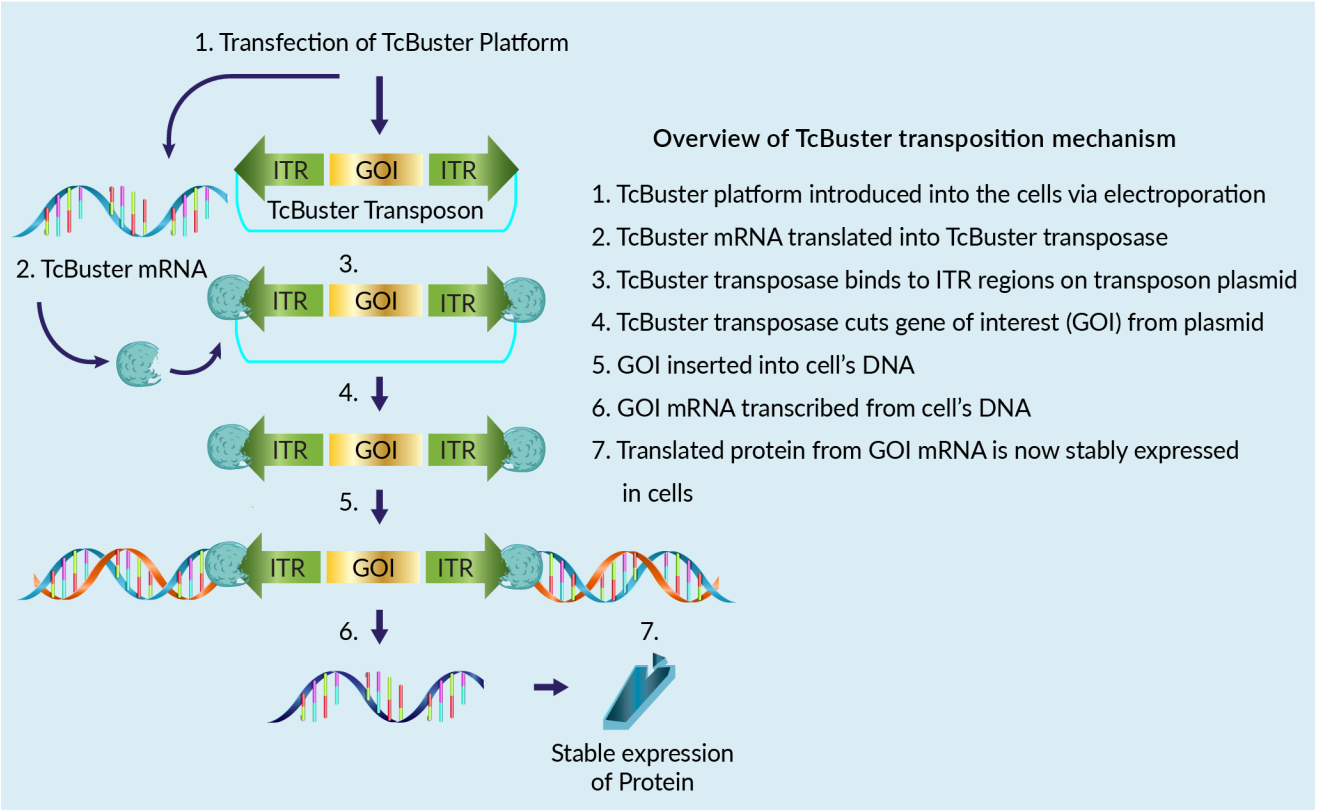
INTRODUCTION TO THE TcBUSTER TRANSPOSON SYSTEM

The TcBuster transposon system for cell therapy applications incorporates gene engineering as well as expression, verification, and target validations. TcBuster is a non-viral gene editing platform aligned for stable integration of a gene of interest (GOI) into the genome. The transposition mechanism is shown in Figure 1.

TcBuster is a part of the hAT family, one of the largest families of transposases found in nature. Successful transposon elements

FIGURE 1

Overview of TcBuster transposition mechanism to produce stable expression of protein.



proliferate and diversify by vertical propagation within a single host species lineage and by horizontal transfer between species. Transposases have evolved with limited activity and mechanisms for regulation, to avoid genomic instability and excessive toxicity to their host.

TcBuster is active in mammalian cells but has low activity in T cells. Homologous transposons across different species tend to diversify, due to genetic drift and adaptation to the host species. Therefore, we have constructed a highly active TcBuster by incorporating phylogenetically conserved amino acids from related hAT family members.

DIRECTED EVOLUTION OF HYPERACTIVE TcBUSTER

Over one hundred consensus amino acids have been identified, and a random combinatorial library of these mutations was generated. The bulk population was tested before cells were sorted and sequenced for high performing mutants. The TcBuster transposase was manually edited to include amino acids associated with high performance to create TcBuster VE7.

To further increase TcBuster's activity, a high-throughput combinatorial library method was used to screen for hyperactivity. These mutations were DNAse treated and randomly assembled to contain two to three alterations per enzyme. This library of TcBuster mutations was then introduced into cells along with three separate transposons, encoding green fluorescent protein (GFP), hygromycin, and mCherry. It was hypothesized that only a highly active TcBuster could stably integrate three separate transposons. Once the cells had been selected on hygromycin, the cells were sorted based on brightness for GFP and mCherry. The bright-cells were isolated and sequenced to identify individual active TcBuster mutations.

Promising TcBuster mutants were screened in HeLa cells. Most mutants were shown to

DOI: 10.18609/cgti.2022.152

perform better than wild-type TcBuster. TcB-M was shown to be the most active TcBuster mutant identified in the screen, increasing transposition efficiency from 20% to over 60%.

The stringency of the screen was increased in T cells, using a tri-cistronic transposon induced experiment. It was further hypothesized that only a highly active TcBuster could stably integrate large cargo in primary T cells. The mutants were tested in T cells in comparison to VE7. Most mutants performed similarly to VE7, however, TcB-M clearly outperformed others.

TcB-M activity in primary T cells was further analyzed using three separate multi-cistronic vectors. In every instance, significantly higher transposition efficiencies were observed with TcB-M compared to VE7. This data was reproduced multiple times in multiple donors, and as a result, TcB-M is now the leading hyperactive TcBuster for cell and gene therapy. It is currently in use by our Custom Gene Engineering Services and is expected to enter clinical application in the near future.

TcBUSTER IN CELL THERAPIES

Increasingly complex cell therapies require a larger cargo capacity, which renders lentivirus unsuitable on its own. Many people are looking for transposon systems to help integrate larger copies.

The editing workflow for both T cells and natural killer (NK) cells includes activation, transposition, and expansion. In a comparison of transposition versus transduction using lentivirus in T cells, it was shown that TcBuster editing efficacy is as high as that of lentivirus (Figure 2).

Scalability

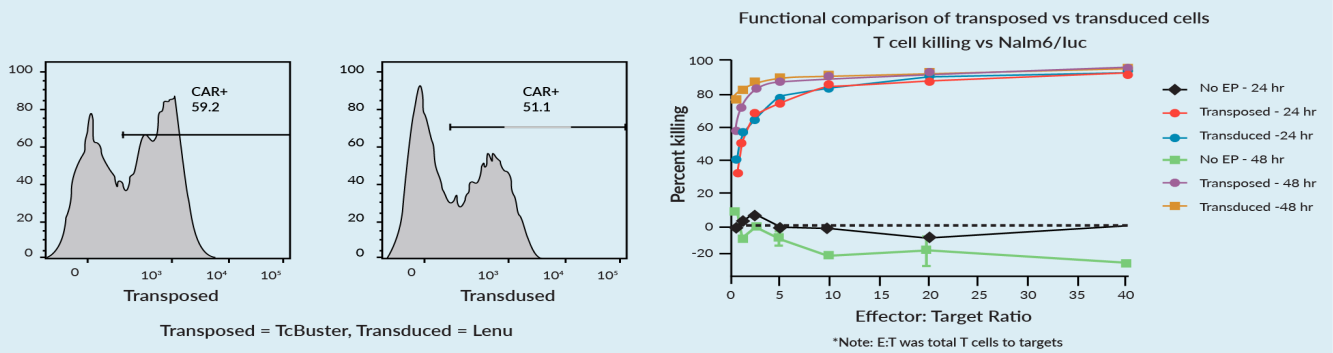
The TcBuster platform can be optimized to support different research and therapeutic scales. TcBuster can be used at small-scale, mid-scale, and large-scale reactions without any drop off in terms of efficiency or cell expansion.

In the small-scale workflow, 10 million cells are electroporated to yield 250–350 million cells in a G-Rex™ 6M plate. At the mid-scale, 80 million cells are electroporated to yield two–three billion cells as an output. The large-scale electroporation of 400 million total cells, yields approximately 15 billion total cells. Folds roughly the same for each of these scales.

Experiments were used to compare their capabilities for transgene expression. A major

FIGURE 2

Comparison of transposed (TcBuster) versus transduced (lentivirus) cells.



- A CD19 CAR is inserted into T cells with TcBuster lentivirus (MOI=5)
- Integration of CD19 with TcBuster is similar to integration with lentiviral methods

- On day 7 post EP, TcBuster and lentivirus edite were incubated with CD19 expressing Nalm6/L
- Similar killing activity was observed with both methods

consideration is to ensure that transposition efficiency is stable at scale. Equal transposition efficiency is found across all scales, suggesting that any difference between proteins in terms of expression is likely due to the detection reagents used rather than scale.

Single electroporation for transposition

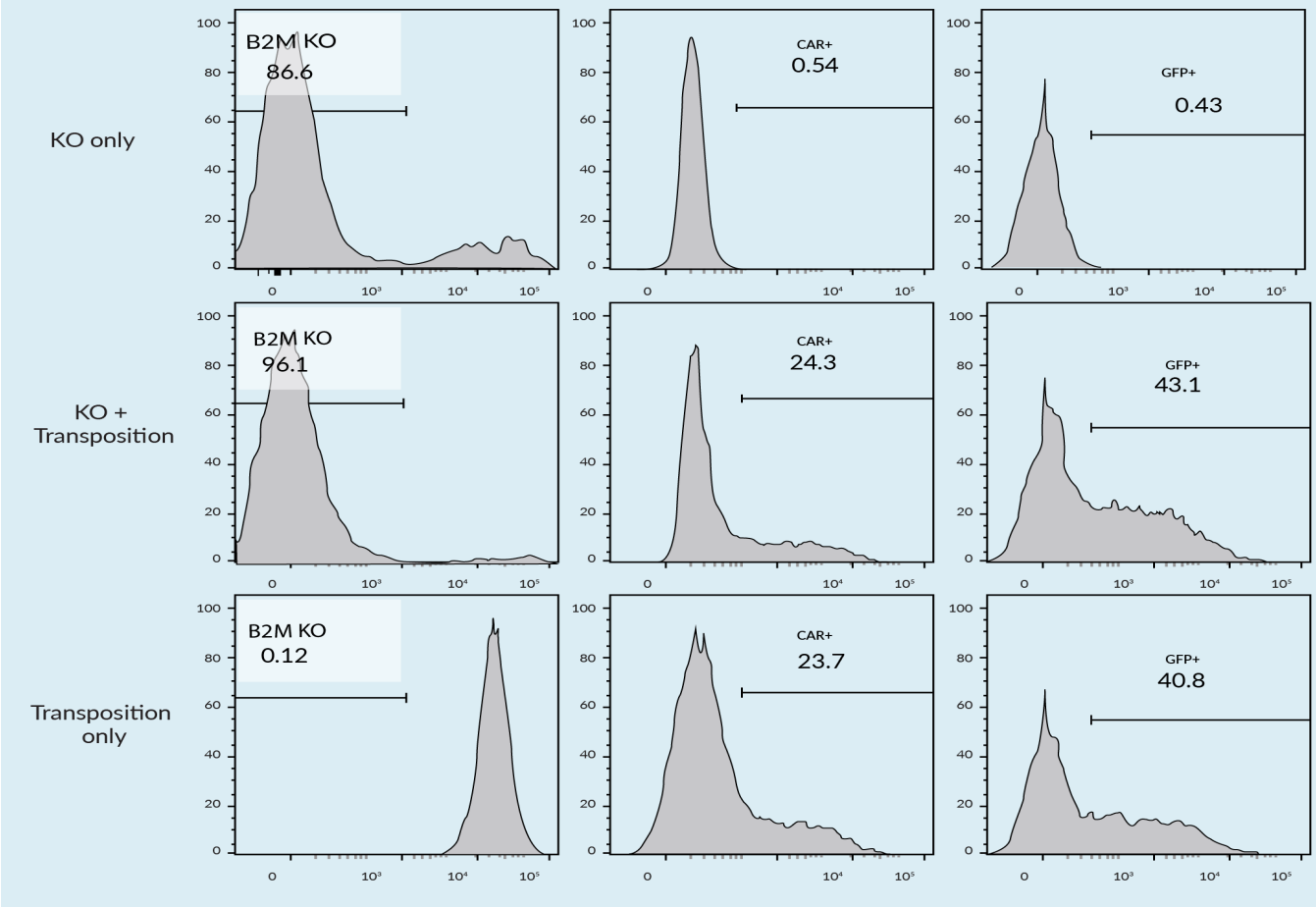
A single electroporation experiment was conducted for both transposition and knockout (Figure 3). Beta 2-Microglobulin knockout was successfully completed with a transposition of a CD19-GFP bicistronic chimeric antigen receptor (CAR). With the TcBuster system, a one-step process can be used to perform knockouts and TcBuster-mediated gene delivery without sacrificing growth and integration efficiency. These results indicate that the addition of clustered regularly interspaced short palindromic repeat (CRISPR) reagents, guide RNA, transposon, and TcBuster reagents, can be performed in a single electroporation, efficiently conducting a knockout and a knock-in, in a single unit operation.

NK cell transposition

CD19 CAR transposition in NK cells has been performed on many donors, and results typically range between 20–40%, respective of the donor. Expansion of transposed NK cells ranges from ~250–1,000-fold in 20 days, dependent upon donor and feeder line. Overall, good expansion has been

FIGURE 3

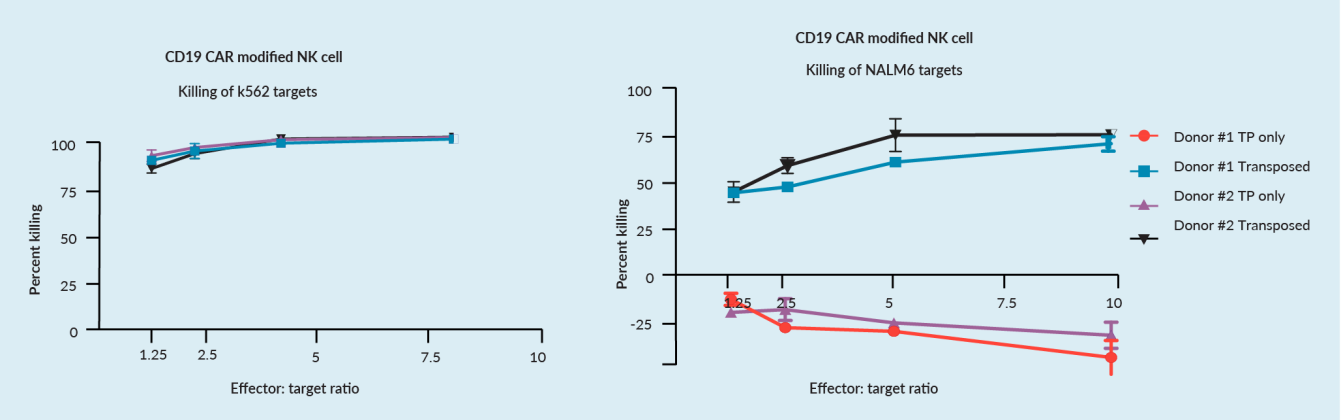
Combination of transposition with beta 2M knockout NK cell transposition.



DOI: 10.18609/cgti.2022.152

FIGURE 4

The augmented capability of NK cells to kill CD19-positive NALM6 luciferase modified targets and K562 targets.



shown with cell viabilities typically greater than 95%.

Transposed NK cells also demonstrated good cytotoxic potential against both K562 cells and CAR-specific killing of CD19-positive targets (NALM6 cells), as shown in Figure 4. It is important that TcBuster does not inhibit the natural killing ability of NK cells, as this adds value in its use within cell therapies.

ADVANTAGES & APPLICATIONS OF TcBUSTER

Target site integration analysis has revealed that the TcBuster transposon system has a safer insertional profile than lentivirus. The median distance to the transcriptional start site was found to be greater than that of lentivirus, suggesting less potential for influencing endogenous genes. Insertional location is less likely to be within intron regions compared to lentivirus. With lentivirus, more exon and intron insertions were seen, with TcBuster locating closer to that of the random theoretical control.

Further experiments were performed to assess the potential for clonality. From an Food and Drug Administration (FDA) regulatory perspective, it is undesirable for a single integration clone to grow out, because it may indicate a transformed T cell. Low frequency of a single integration site indicates that the final cell population has a diverse set of integration events indicating a diverse initially edited population. TcBuster clones showed a diverse integration profile, suggesting a lack of clonal outgrowth.

TcBuster is moving closer to use in the clinical setting. Luminary Therapeutics has filed for an Investigational New Drug application (IND) using TcBuster in the clinic for a B-cell activating factor (BAFF) ligand-based CAR T cell therapy, that targets three receptors expressed in B cell cancers. TcBuster has also been shown an efficient method to engineer and produce primary CAR NK cells targeting CLL-1 for acute myeloid leukemia (AML).

Further, TcBuster can be utilized in induced pluripotent stem cell (iPSC) applications. The platform was implemented in a gene replacement project to engineer iPSCs with a gene under a constitutive promoter. The median distance to the transcriptional start site and insertion location are similar in iPSCs and primary T cells. The desired clones can be selected via genetic screening.

Other applications of TcBuster include increasing antibody production titer, while simultaneously reducing manufacturing timeline. Using TcBuster internally at Bio-Techne, a 32-fold increase in CD3 production and a 58-fold increase in CD28 expression was achieved. TcBuster has also been utilized to generate a K562-based stable feeder cell line for primary NK cell culture needs. It can also generate stable cell lines for drug discovery

workflows, either as population or high-expression clones.

FUTURE DIRECTIONS

Bio-Techne intends to provide TcBuster as a protein. Additionally, we strive to continually enhance the TcBuster activity profile, to more efficiently deliver larger packagers. Another goal is to leverage the properties of TcBuster as a fusion protein. GFP can be fused to the TcBuster without any loss in transposition efficiency, which enables the potential of improving its integration profile and specificity.

Q&A

David Hermanson (left) and **Scott Silaika** (right), Director, Commercial Business Development, Cell and Gene Therapy, Bio-Techne, answer questions about the TcBuster platform.

Q Is there a GOI size limit for TcBuster, and is the integration site-specific?

DH: The size limit for TcBuster is based on the transfection technologies. We found that electroporation generally cannot get a plasmid larger than 10–12kb into the cell. TcBuster itself can transfer large cargoes over 10–12kb. However, practically speaking, when transfecting T cells or NK cells, getting plasmids much larger than 10–12kb starts to become a challenge.

The integration is not site-specific. It is a random integration, though we do have thoughts on how to skew that integration profile and could eventually perhaps make it site-specific.

Q How does TcBuster compare to other non-viral systems such as Sleeping Beauty or PiggyBac?

DH: We have done some limited direct comparisons between TcBuster and Sleeping Beauty and have found that the TcBuster does have higher integration rates than the Sleeping Beauty SB100. We have never done a direct comparison to piggyBac to date, especially with some of the hyperactive mutants that are being utilized by Poseida Therapeutics.

DOI: 10.18609/cgti.2022.152

We could compare it to the literature data of integration profiles. Out of the three transposons, Sleeping Beauty is more random than TcBuster. PiggyBac is slightly more preferential to active chromatin. All of them show more random integration than a lentivirus.

Q How many copies are integrated into the genome and what is the variability in copy number from cell to cell?

DH: We have done copy number analysis using droplet digital PCR. For our populations, our copy number is found to be around three–five in T cells, and slightly lower in NK cells at two–three copies per cell range in terms of the entire population. With T cells and NK cells, we have not analyzed individual cells to evaluate copy numbers, so we do not currently have the data on variability.

Q How can TcBuster be evaluated by interested parties?

SS: There are two methods to start evaluation. One would be a proof-of-concept study at Bio-Techne, where we gauge with potential clients and test their cells using TcBuster, providing the final frozen cells, the protocol, and the test results.

The other is a material transfer agreement, where we provide the transposase and either standard transposons such as CD19 or GFP, or custom transposons with the GOI. This can be done quickly and relatively inexpensively for proof-of-concept.

Q Is there a license fee for the use of TcBuster?

SS: There is a fee. The business model has a few different components, and is milestone-based, as the clients progress through the clinic. There is a royalty associated with the use of a commercial basis. We try to keep it reasonable up front, until both our customers and eventually, ourselves will be successful commercially. We are happy to discuss this further.

Q Has TcBuster been used in the clinic?

SS: Our first clinical use will be soon, within a matter of months. The IND is already approved.

Q Have you tried any transfection technologies other than electroporation?

DH: We are always interested in evaluating the squeeze technologies. We have investigated some of the other technologies such as the SE cell Line or Tito Pen, as well as lipid nanoparticles.

We do not have any concrete data that I am able to share, but it certainly is something that we have hopes for. One of the major limitations in terms of getting the cargo in and the size of the cargo comes down to the transfection technology. If we can move away from electroporation into another transfection modality that allows for larger plasmids, then our ability to transfer even larger cargoes will improve.

Q Does Bio-Techne offer off-the-shelf TcBuster GFP to test or use as a control for initial feasibility studies?

SS: We have standard CD19 GFP constructs that we can provide quickly.

Q How do you assess days post-NK activation for best transposition?

DH: Normally, our transposition rates in NK cells are most dependent on the method of growth. We tend to try to think up our transgene expression with the activation. For example, if we are using K562s as a feeder line, stimulating them every 7 days, typically we will look at the transgene expression one–two days after stimulation. You will see some variability seven days after stimulation, and the transgene expression percentage will be lower than two–three days after stimulation. That holds true through multiple rounds of stimulation. There is part of the cell cycle that comes into play in terms of detecting it.

Q How would you say this approach compares with the latest advances in the lentiviral space for CAR T cell generation?

DH: In terms of the phenotype for transposed cells versus transduced cells, in general, transposon systems lead to a higher percentage of memory cells. This is related to how the mechanism of transposons perform better in naïve cells than in the effector populations from the apheresis product.

Q Have you compared with CRISPR/Cas9, and if so, are there any advantages with TcBuster?

DH: We have not a done head-to-head comparison, though we have worked with customers who are evaluating them side by side. The integration rates tend to be higher using TcBuster than with a CRISPR/Cas9 directed site integration. The CRISPR/Cas9 mechanisms work acceptably for relatively small cargo. However, as you move to larger cargo, we have seen the rates fall off significantly.

DOI: 10.18609/cgti.2022.152

Q Does copy number increase with cell propagation, or is it limited by turnover of transposase?

DH: Transposition occurs in the first 48–72 hours after electroporation. After that point, the transposase mRNA as starts to become undetectable by a qRT-PCR reaction. After the first 72 hours, there is no more transposition occurring, so the copy number does not continue to increase.

The integrations are also stable long-term. We have taken T cells and continued to activate them through CD3/CD28 bead activation to the point where the T cells no longer expand *ex vivo*; we still maintain the same level of integration throughout. There is no difference in copy number whether you run it 7 days post-transposition or after three–four rounds of stimulation.

Q Can you clarify why GFP affects Sleeping Beauty but not TcBuster mechanisms?

DH: Some people have tried to fuse GFP onto both the N terminal and C terminal of Sleeping Beauty, and it has been shown to prevent all transposition activity. We have been able to fuse GFP to TcBuster and still get equal transposition efficiency. It seems to be an inherent property of the enzymes.

Q What is the cost of transposition versus LV transduction per sample?

DH: In a clinical-scale manufacturing run, the cost of lentivirus can be anywhere from about $15,000–$25,000 depending on lentivirus run efficiency. We are targeting <$10,000 per patient cost of goods for both the transposon and transposase.

AFFILIATIONS

Xiaobai Patrinostro PhD
Manager of Genome Engineering Services Cell and Gene Therapy,
Bio-Techne

David Hermanson PhD
Senior Manager of R&D Applications in Cell and Gene Therapy,
Bio-Techne

Scott Silaika
Director,
Commercial Business Development, Cell and Gene Therapy,
Bio-Techne

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 Hermanson is a Bio-Techne stockholder.*

Funding declaration: *Dr Patrinostro received SBIR grant solicitation # PA17-302; awarded 2019-06-01 to 2021-05-31.*

ARTICLE & COPYRIGHT INFORMATION

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

Webinar recorded: *Jun 16 2022;* ***Publication date:*** *Sep 26 2022.*

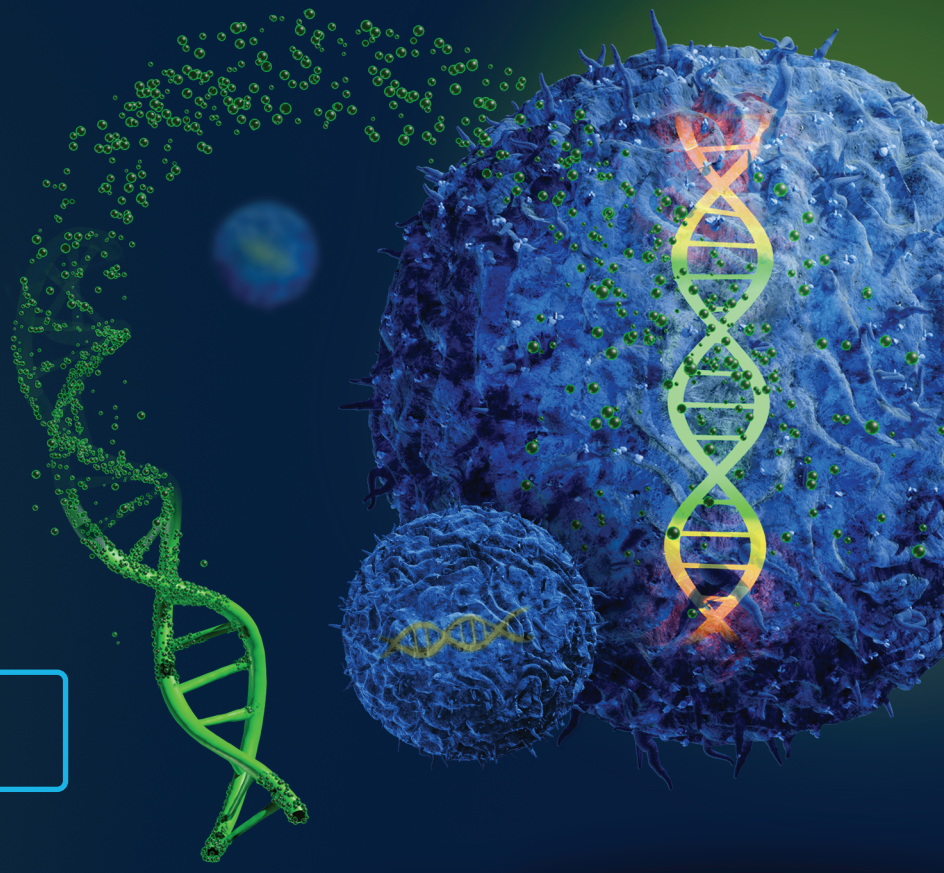
DOI: 10.18609/cgti.2022.152

bio-techne®

Gene Engineering with TcBuster™

Non-Viral Transposon System

- Stable, High-Efficiency Integration
- Shorter Timelines at Lower Cost
- Large Cargo Capacity
- Multiple Edits in One Step



Learn More

Successful suspension-based viral vector manufacturing scale-up from process development to clinic

Denis Kole, PhD, Pall Corporation, **Timothy P Cripe**, MD, PhD, Nationwide Children's Hospital & The Ohio State University College of Medicine, **Lenore Giannunzio**, Andelyn Biosciences, & **Cassie-Marie Peigné**, PhD, Polyplus

Cell and gene therapies have emerged as promising treatments for previously untreatable diseases, with viral vectors being used as the current preferred delivery vehicle. Safe, robust, and cost-effective vector manufacturing processes are necessary to support the demands of the patient population. Here, expert therapy developers, solutions providers, and CDMOs discuss considerations for the successful scale-up of viral vector processes from bench to commercial manufacturing.

HOW TO MITIGATE THE CHALLENGES FACED WHEN BRINGING A PRODUCT TO COMMERCIAL MANUFACTURING?

"People involved in the AAV field exist in concentric rings **(Figure 1)**. A CDMO must know that clients and users sometimes lack the information to know what is possible or expected at the agency level. As a client, I rely on a CDMO to educate me about best practices and CMC issues. Early collaboration with a CDMO to discuss these points from the get-go is key to making informed decisions. Delayed learning can lead to the need to backtrack, which can be very costly and time-consuming."

- Timothy Cripe, Nationwide Children's Hospital & The Ohio State University College of Medicine

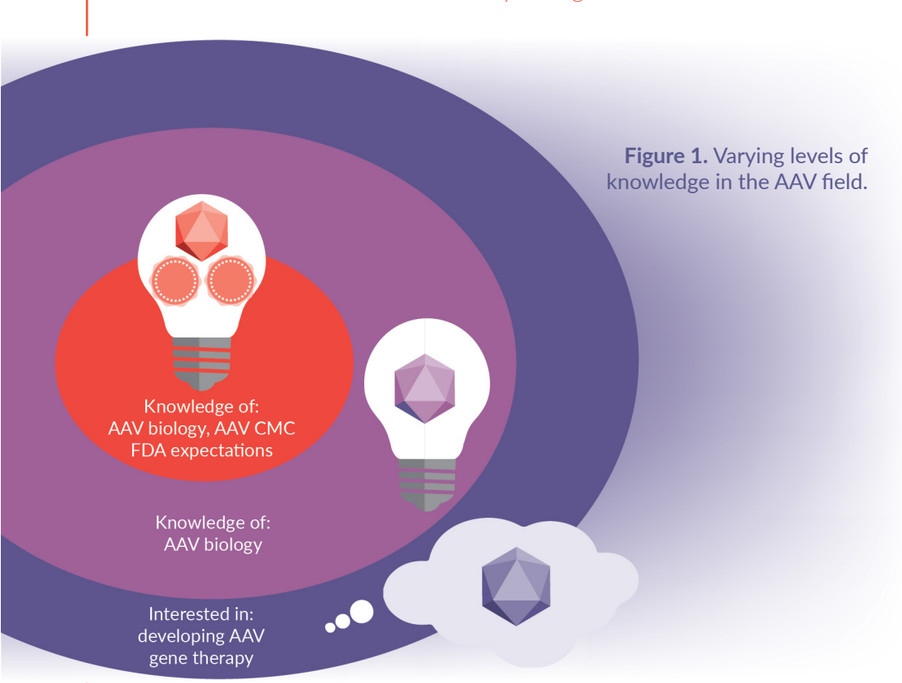


Figure 1. Varying levels of knowledge in the AAV field.

WHAT ARE THE KEY CONSIDERATIONS WHEN PLANNING FOR LONG-TERM SUCCESS IN SUSPENSION-BASED SCALE-UP?

"Design for quality as well as productivity. Selecting an optimal production system, including reagents and equipment, is extremely important, and will have huge implications on the roadmap to commercial manufacturing. Design and develop a small-scale process that fits the target manufacturing-scale operation, to avoid large potential shifts in operating parameters and technology changes during scale-up."

- Denis Kole, Pall Corporation

WHERE ARE WE SEEING PROGRESS IN BOOSTING YIELDS IN UPSTREAM VIRAL VECTOR PROCESSING?

"From a provider of transfection solutions perspective, we have worked to create transfection reagents designed for current systems in suspension production to ease process scalability. We are constantly innovating to provide new solutions to ease scalability and boost yields of all parts of the upstream process, from the cell line to the design of bags and bioreactors."

- Cassie-Marie Peigné, Polyplus

HOW ARE PROCESS AND ANALYTICAL TECHNOLOGIES HELPING TO DELIVER FLEXIBILITY TO ENABLE OPTIMAL SCALE-UP?

"It can take a long time to develop analytics, for example digital droplet PCR, and then transfer that technology to the CDMO. Flexibility for CDMOs to use their own internal analytics during process development, such as a standard PCR for quantification, can speed up figuring out the scale-up process."

- Timothy Cripe, Nationwide Children's Hospital & The Ohio State University College of Medicine

HOW CAN WE ACCELERATE THE SCALE-UP PROCESS WITHOUT COMPROMISING ON ROBUSTNESS?

"We can integrate the clients' needs with novel solutions developed by vendors and technical accelerators and pull this all together with managing and regulatory expertise. By troubleshooting at a small scale, hurdles can be solved faster. This also allows time to complete engineering runs to reduce scale-up risk and provide additional vector for assay qualification."

- Lenore Giannunzio, Andelyn Biosciences

HAVE YOU OBSERVED A PREFERENCE WITHIN THE FIELD BETWEEN ADHERENT AND SUSPENSION PRODUCTION PLATFORMS?

"To date, the adherent approach has proven to be successful with gene therapies currently on the market targeting rare diseases. Over the next few years, we will continue to see more of a mix of both adherent and suspension platforms being utilized. As the field continues to evolve, it is likely that suspension-based processes will become the industry standard for viral vector manufacturing."

- Denis Kole, Pall Corporation

TOP TIPS FOR SUCCESSFUL SUSPENSION-BASED VIRAL VECTOR MANUFACTURING SCALE-UP

"Plan for scalability as early as possible in the process."

- Denis Kole, Pall Corporation

"In research, use products that you know will be available in a GMP setting."

- Lenore Giannunzio

"Find a good scale-down model to optimize your parameters."

- Timothy Cripe, Nationwide Children's Hospital & The Ohio State University College of Medicine

Cell & Gene Therapy Insights 2022; 8(8), 1215. DOI: 10.18609/cgti.2022.180

CELL & GENE THERAPY INSIGHTS

CELL & GENE THERAPY MANUFACTURING SCALE-UP/SCALE-OUT

SPOTLIGHT

LIVE30 TRANSCRIPT

Process development optimization for GMP manufacturing: a CAR-T case study

Gary M Pigeau

The cell and gene therapy industry is revolutionizing how we approach previously intractable diseases. The pipeline of these therapies is full of promise for durable cures, but the necessary tools, processes, and end-to-end solutions are required to bring these emerging therapies to the clinic.

The path to commercialization can vary depending on the type of therapy being developed. However, all therapeutics go through similar milestones: starting at discovery and working towards commercial manufacturing. This article will focus on the middle of that journey with process development and clinical manufacturing. Specifically, on an oncology application with integrated aspects of gene therapy development and manufacturing. A chimeric antigen receptor T cell therapy PD journey towards GMP manufacturing will be outlined, with a focus on Cytiva's collaboration with the Centre for Commercialization of Regenerative Medicine (CCRM) in Toronto.

Cell & Gene Therapy Insights 2022; 8(8), 1445–1452

DOI: 10.18609/cgti.2022.210

PEOPLE, FACILITY & AGREEMENTS

As you engage with a contract development manufacturing organization (CDMO) on this journey to the clinic, there are some considerations to keep in mind. Your first interaction is likely to be with someone from business development. This should be followed by conversations with a subject matter expert who can engage the developer's experts in technical discussions. These discussions should be transparent and collaborative, with any risks

being well-communicated and understood by both parties.

Choice of facility is an interesting consideration – do you choose one that is set up strictly for manufacturing with many clean rooms? Or do you select a site with process development (PD) and clean room capabilities? A further consideration is the stage of asset development. In preclinical or early-phase manufacturing, there are opportunities for continuous improvement programs, so a facility that offers both PD and good manufacturing practice (GMP) manufacturing is beneficial. The agreements required for this arrangement are the confidential disclosure agreement, the master services agreement, the quality agreement, and the scope of work (SOW) – which includes the project change order and the material transfer agreement.

A CAR-T CASE STUDY

Chimeric antigen receptor (CAR)-T cell therapy is an on-market, autologous form of cancer immunotherapy. In the manufacturing process, the patient's blood cells are purified, selected, and sorted, before being genetically modified – typically with a lentivirus (LV) – to express a CAR allowing the T cells to target cancer cells. These modified cells are then infused back into the patient. The manufacturing time can typically take between 2–4 weeks.

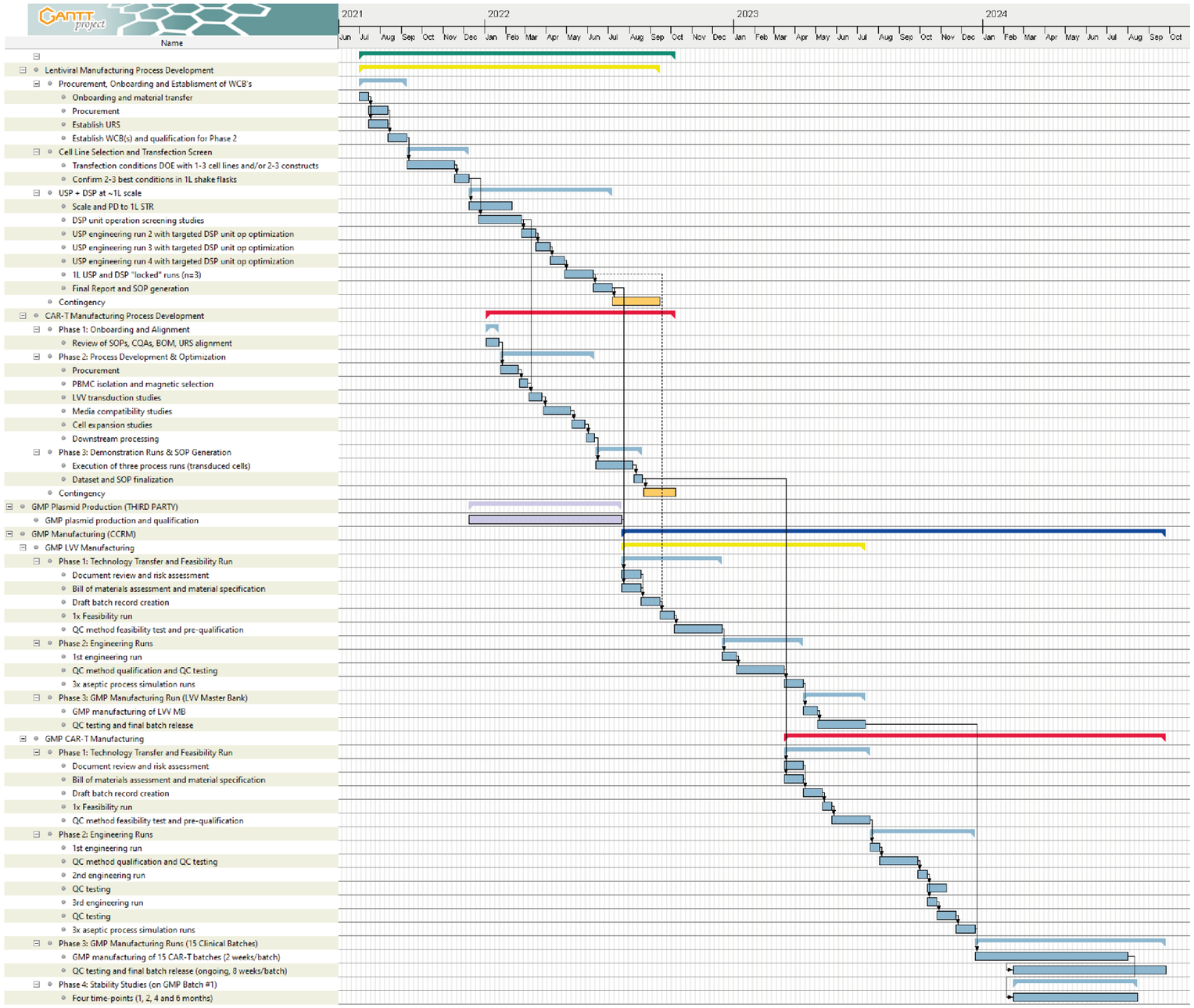
The assumptions in this case study include that a customer has already established a proprietary CAR and that they have a handful of host cell lines for the lentivirus. The work done in the customer's lab has been performed on a small scale in well plates or T-flasks and has shown promise in various models. They have the funding to move ahead with PD and the manufacturing of this asset.

Lentivirus process development (LV PD)

In this case study (Figure 1), the LV PD has an initial phase of onboarding of materials, procurement, and alignment of the user-requested specifications. In this instance, the

FIGURE 1

A CAR-T case study timeline including LV PD, cell therapy PD, LV GMP manufacturing, and cell therapy GMP manufacturing.



WCB: working cell bank; URS: user requested/required specification; USP: upstream processing; DSP: downstream processing; DoE: design of experiment.

customer has a handful of cell banks to be developed into working cell banks and qualified for the second phase. Phase two focuses on cell line selection and screening of transfection conditions with one to three cell lines and multiple constructs. The plasmid-to-transfection reagent ratio is one of the key factors in optimizing transfection. Achieving this across multiple cell lines with multiple constructs is a substantial task. This can be simplified by reducing the numbers of these inputs. Once those preliminary screens are complete, the best two to three conditions are then scaled up into shake flasks before phase three, during which the conditions are moved into 1 L stirred tank bioreactors to feed the downstream optimization. This happens in parallel, to gain a head start on the downstream optimization.

Once this process is complete and the desired operating space has been identified, the engineering runs begin. Three runs have been budgeted to form the basis for the standard operating procedure (SOP), which is then transferred to GMP colleagues for manufacturing and translation into the batch record. With any development program, we include some contingency. If a customer is coming in with a proprietary CAR and some cell lines, the estimated timeframe is roughly a year, though it can be more or less than that.

CAR-T manufacturing PD

As soon as the team has the modified virus from the first SOW, we begin using it in transduction studies for CAR-T. Phase one will include a review of the procedures, the critical quality attributes and the bill of materials, as well as aligning the user-requested specifications. The individual tasks in Phase two can be optional, depending on the background development performed by the customer.

Typically, we begin with cell isolation and selection, then move on to transduction studies to determine the appropriate multiplicity of infection (MOI) for the cell type. We want to minimize the MOI to minimize viral cost whilst optimizing transduction. Media compatibility is another factor to consider, as it takes time to select an optimal medium. Cell expansion studies typically define how these cells grow and respond within automated bioreactors. Downstream processing is another automated unit operation provided by Cytiva.

FIGURE 2

The FlexFactory™ platform.

What makes a FlexFactory™ platform?

Cytiva cell therapy instruments

- Most cell therapy products are manufactured with Cytiva instruments
- Closed, automated, and scalable manufacturing solution
- Third party process equipment

Qualification and documentation package

- Provides documentation to help meet regulatory requirements
- Experienced team builds documents for you with regulators in mind

Personal liaison

- Single point of contact for project
- Dedicated project team consisting of Cytiva subject matter experts
- Staging, resource allocation, and coordination
- On-time project turnover

Consulting and training

- 10 full days of in-person/virtual staff training including onboarding for future employees
- Address pain points and knowledge gaps
- Build cGMP competences
- Process economics analysis

Chronicle™ automation

- Manufacturing Execution System (MES) digital automation platform
- Meets all global regulatory requirements
- eSOP, eBMR, instrument data capture
- Reduces paper-based documentation processes

In phase three of the CAR-T PD, we have three process runs that are locked in, using transduced cells. Consistent running of the process provides the data to finalize the SOP, which is passed on to the customer's GMP facility.

LV GMP manufacturing

In this case study, the first phase of technology transfer and feasibility was completed at Centre for Commercialization of Regenerative Medicine (CCRM) in their GMP manufacturing facility. Phase one included documentation and risk assessments of the incoming processes, with protocols from LV PD feeding into GMP. Next, a feasibility run is typically executed, either in-suite or out-of-suite. Work then commences on the quality control (QC) method feasibility and prequalification to enable later application of these methods in manufacturing for clinical use.

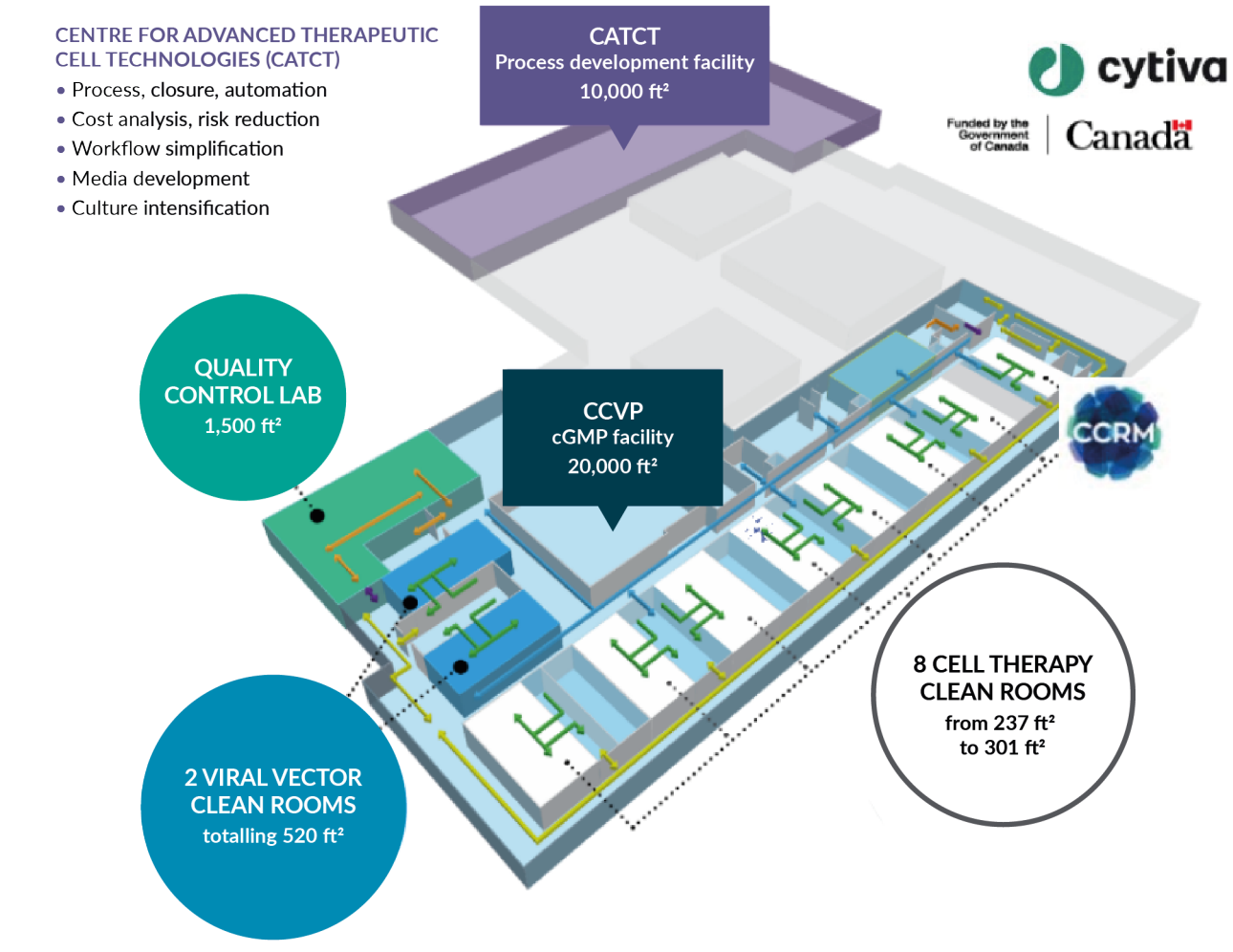
In phase two, qualified and tested engineering runs are executed under GMP conditions through execution of a batch record. Aseptic process simulation runs also take place, which ensure the process is sterile. At the end of this GMP manufacturing run for the LV itself, the manufacturing of LV master banks is tested, and the batch is released.

CAR-T GMP manufacturing

The final stage is the manufacturing of the CAR-T therapy itself, culminating in a phase one clinical trial. This begins with the

FIGURE 3

Fast Trak Center, CATCT and CCVP at CCRM, Toronto.



DOI: 10.18609/cgti.2022.210

document review, bill of materials, batch record, and feasibility run, before moving into engineering. Typically – as this process will be used in the clinical trial – multiple runs will be suggested by the manufacturer. The goal is to demonstrate the consistency of the product and process before making the clinical batches. In phase three, a timeline of two weeks per batch manufactured is assumed. QC and final release of that batch will then take around eight weeks per batch, before final stability studies are conducted.

From engaging with the CDMO to manufacturing in a clinical trial, a timeline of two and a half years is proposed. This is dependent on how you interface with your CDMO, the current state of your technology, and what needs to be developed and characterized.

CYTIVA SOLUTIONS

At Cytiva we offer Enterprise Solutions for cell therapy to assist customers in scaling up or scaling out to prepare for clinical trials or commercialization. Our equipment enables functionally closed, automated, and compliant manufacturing workflows, and the equipment is flexible – it can support multiple cell therapies. Our mission is to propel you to your next milestone and into the clinic. We want you to come to the market at speed and with the strength to deliver for your patients and your investors. The three pillars of Cytiva's Enterprise Solutions include the FlexFactory™ platform, Fast Trak™ services, and the KUBio™ facility to provide a comprehensive solution including equipment, integrated services, and staff training to solve challenges encountered during scale-up, scale-out, manufacturing, and site expansion.

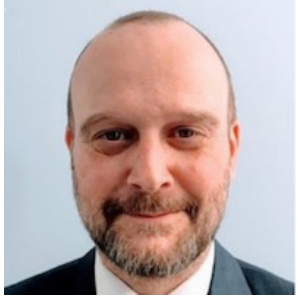
Our FlexFactory™ platform **(Figure 2)** includes manufacturing hardware, qualification, and documentation packages for GMP-compliance, project management and oversight, and consulting and training for your staff and operators. The entire manufacturing process can be overseen by our Chronicle™ manufacturing execution system.

The Fast Trak Center in Toronto is a collaboration between Cytiva and CCRM, including the Center for Advanced Therapeutic Cell Technologies (CATCT) and the Center for Cell and Vector Production (CCVP), for a variety of cell and gene therapy manufacturing needs **(Figure 3)**.

CONCLUSION

In summary, it is important to choose a partner experienced in both the science and manufacturing of advanced therapies. Keep in mind the initial time that is required to establish all the agreements that are going to define your journey. Be transparent about the GMP-readiness of your technology, and if you are not ready for manufacturing, choose a partner with PD facilities and experience with technology transfer.

Q&A



David McCall, Editor, BioInsights speaks to Gary M Pigeau PhD, Director of Research and Development, Nucleic Acid Therapeutics, Cytiva (pictured)

Q How would timelines be affected if a customer already had their LV process sorted out?

GP: There are four stages of development here, with two of them dedicated to the LV process. Those could be reduced if there was a batch record and an existing process that had already been validated. You could shave 6 months off the LV process development. Then, we would use the process to manufacture from a PD scale, manufacture some research use-only virus, and feed that directly into the CAR-T PD whilst completing the LV GMP manufacturing in parallel.

Q How important are closed, automated, and scalable processes for PD projects?

GP: Moving a process into a bioreactor takes work, and the sooner that you consider automated, closed, and scalable implementation, the better. Current GMP manufacturing still uses open processes, which is reflective of the maturity of the industry. As we advance, processes will move towards being closed, aside from a few required operator interventions. The sooner you can demonstrate compatibility with closed and automated systems, the better it is for transitioning into manufacturing. It might take a little more work upfront, but it is helpful for making that transition into the clinic.

BIOGRAPHY

Gary Pigeau received his PhD in Biotechnology from Brock University in St. Catharines, Ontario. He completed a postdoctoral fellowship at the University of Alberta with a visiting scientist appointment at the University of Oxford. Gary moved to private industry in 2008, where his research group focused on process development and scale-up of a proprietary bioprocess technology. He then moved to commercial, large-scale biomanufacturing in 2013. Gary joined Cytiva, formerly part of GE Healthcare's Life Sciences business, and the Centre for Advanced Therapeutic Cell Technologies (CATCT) at CCRM in 2016. He served until mid-2022 as the Director of the collaborative facility in Toronto, Ontario with focus on

DOI: 10.18609/cgti.2022.210

biology support for New Product Introductions, internal technology development projects and contract process development services in cell and gene therapy. Recently, Gary has taken a new role with Cytiva as Director, Research & Development for the new Nucleic Acid Therapeutics business.

AFFILIATION

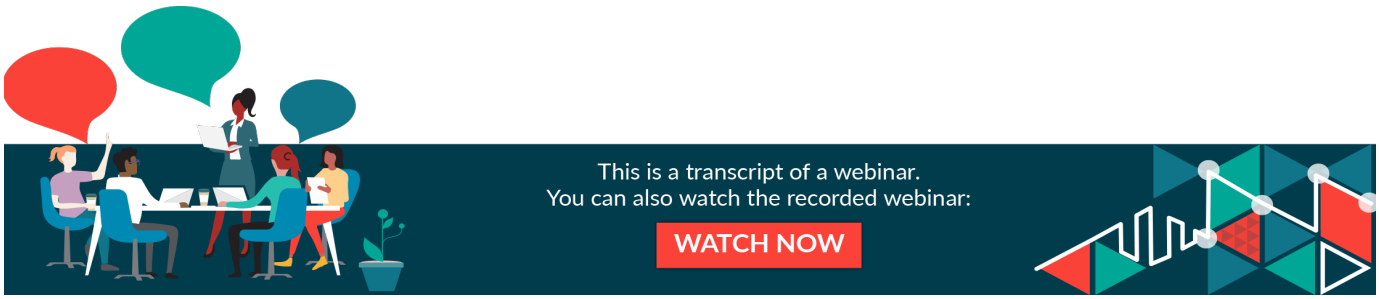
Gary M Pigeau, PhD
Director of Research and Development,
Nucleic Acid Therapeutics,
Cytiva

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *The named author takes responsibility for the integrity of the work as a whole, and has given his 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 author received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: This article was written based on a webinar entitled Process development optimization for GMP manufacturing: A CAR-T case study which was held on Oct 6 2022.
Webinar recorded: Oct 6 2022; **Revised manuscript received:** Nov 16 2022; **Publication date:** Nov 9 2022



Refine your process and maintain control

Optimize and scale your PD process, while maintaining your IP

cytiva.com

For local office contact information, visit cytiva.com/contact

CY30748-31Aug22-DF

FASTFACTS

Off-the-shelf lentiviral vector packaging plasmids

James Cody, Associate Director, Technical Sales and Evaluations, Gene Therapy CDMO Services, Charles River Laboratories

Challenges in plasmid sourcing can jeopardize timelines for advanced therapies. Off-the-shelf packaging plasmids can help to streamline the path to plasmid production for cell and gene therapy uses. Charles River Laboratories offers off-the-shelf plasmids to comprise part of their end-to-end support for advanced therapies.

PLASMID DNA PRODUCTION

Many advanced therapy approaches are dependent on plasmid DNA (pDNA) as a critical starting material. However, some pDNA production challenges must be overcome, such as production and capacity bottlenecks, cost and timeline pressures, and a need for attention to process development and optimization. To future-proof your plasmid strategy, Charles River offers an efficient and robust plug-and-play development toolbox with phase-appropriate platform production, purpose-built facilities and quality systems, and off-the-shelf products.

OFF-THE-SHELF PLASMID PRODUCTS

Fully custom plasmid manufacturing can increase lentiviral vector production process lead time, cost, and complexity. However, there are common viral vector packaging plasmids **(Figure 1)**, many of which are relatively universal, meaning off-the-shelf availability is possible. Off-the-shelf packaging plasmids produced by standardized methods offer the advantages of being immediately available, reducing development costs and simplifying supply chains. They can enable streamlined and secure plasmid production and a stable supply of cell and gene therapy products.

Figure 1. Common viral vector packaging plasmids.

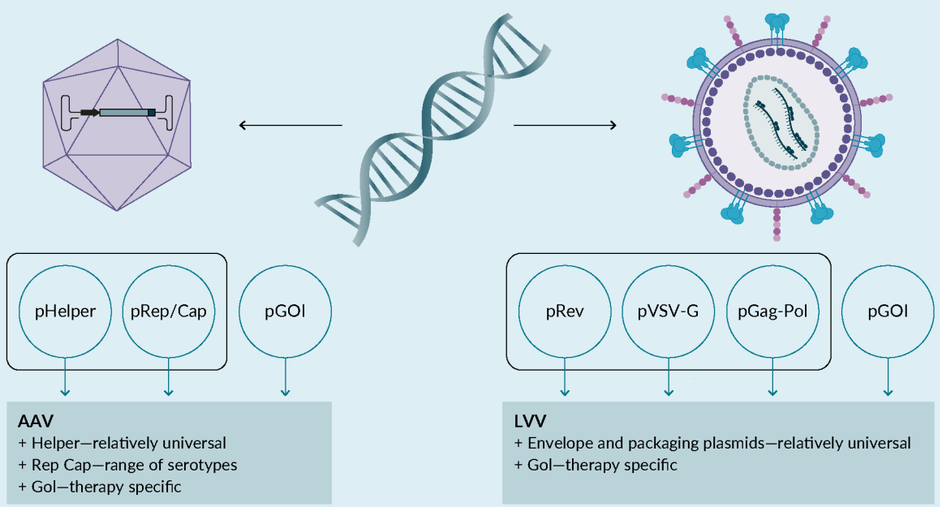
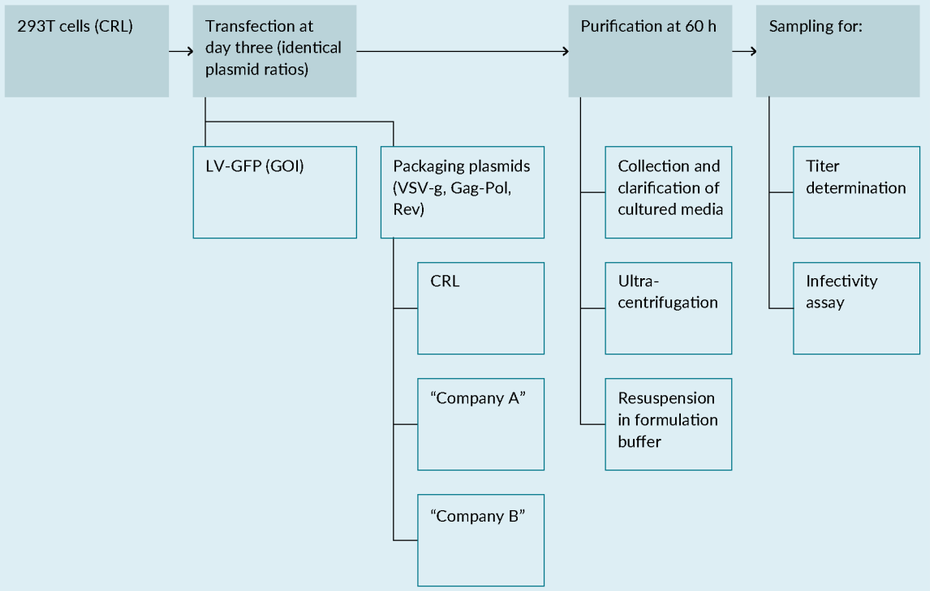


Figure 2. Summarized protocol of the packaging plasmid comparison study.



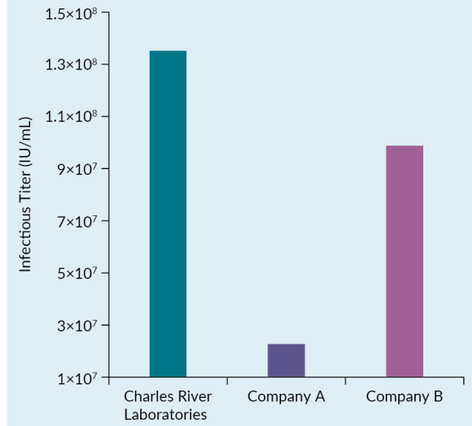
Charles River's planned portfolio of off-the-shelf plasmid products consists of adeno-associated viral (AAV) vector helper plasmids and several rep/cap variants, as well as lentiviral Rev, Gag-Pol, and VSV-G in both high quality (HQ) and GMP grades. These are based on GMP master cell banks and are all subjected to a standard testing panel. In addition, pipeline products include antibiotic-free ORT plasmids and generic backbone plasmids for linearization to be used for mRNA templates.

PACKAGING PLASMID COMPARISON STUDY

Charles River Laboratories' plasmids were compared with those from two other suppliers in small-scale lentiviral vector production. The protocol is summarized in **Figure 2**. HEK293T cells were transfected at day three using identical plasmid ratios, with lentiviral-GFP gene of interest (GOI) plasmids and three packaging plasmids, sourced either from Charles River, Company A, or Company B. Samples from each experiment were tested by standard infectivity assay using a cell-based method and qPCR for titer determination.

As shown in **Figure 3**, Charles River's lentiviral packaging plasmids gave yields of infectious lentiviral vector comparable to those of two other commercial suppliers. The potency of Charles River's lentiviral packaging plasmid was confirmed by infectivity testing.

Figure 3. Confirmation of lentiviral infectivity across plasmid groups.



Cell and Gene Therapy Insights 2022; 8(8), 2001; DOI: 10.18609/cgti.2022.237

INTERVIEW

Beginning with the end in mind: how early plasmid design and CDMO partnership can set you up for success in cell or gene therapy manufacturing

Roisin McGuigan, Editor, *BioInsights*, talks to OXGENE's **Richard Parker-Manuel, PhD**, Group Leader for Plasma Engineering & Production &, **Qian Liu, PhD**, Head of Engineering & Production

RICHARD PARKER-MANUEL, PhD, is a Group Leader for Plasmid Engineering and Production at OXGENE, A WuXi Advanced Therapies Company. He has twenty years' experience in molecular biology with nearly seven years in the cell and gene therapy space. He oversees the design and engineering of adeno associated virus and lentivirus vector plasmids for a variety of clients. He is also involved in several R&D initiatives with the aim of making OXGENE's excellent vectors even better.

QIAN LIU, PhD, joined OXGENE in July 2017 as a Cell Line Engineering Scientist. She has a background in cell and molecular biology, She was then gradually promoted to lead all biomanufacturing services OXGENE provide. Prior to joining OXGENE, Qian was a postdoctoral researcher in the field of Regenerative Medicine, involved in bioartificial liver development and stem cell differentiation mechanism investigation in Loughborough University and the University of Nottingham, respectively. Qian obtained her PhD in Molecular Nutrition from the University of Nottingham.

Cell & Gene Therapy Insights 2022; 8(8), 945–951

DOI: 10.18609/cgti.2022.140

Plasmids are important tools for building gene therapies, but substantial challenges exist for manufacturing a plasmid-based therapy – making it crucial for innovators to be aware of best practises. We spoke to Dr Richard Parker-Manuel and Dr Qian Liu about how careful plasmid design through promoter/capsid discovery and plasmid engineering early on in therapeutic development can help ensure that an innovator begins their journey with the right tools – and how an early CDMO partnership can foster success later down the line.

Q Could you both tell me a bit about your backgrounds, and your current work?

RP: My background is in DNA replication, recombination, and repair. For my PhD in Bath, I researched DNA replication in archaea before moving to York to do a post-doctorate working on mini-chromosome maintenance protein (MCM), the human replicative DNA helicase complex. I first joined OXGENE in 2012. I currently run the plasmid engineering and production group, in which we make constructs and libraries for a variety of commercial and R&D projects. The work we are currently doing includes assembling libraries for promoter and capsid screening platforms, improving our transient lentiviral packaging plasmid system, and assembling constructs for customers who wish to evaluate our TESSA™ adeno-associated virus (AAV) manufacturing technology. We are also setting up a manufacturing facility for research grade AAV and lentiviral plasmids.

QL: I did my PhD in molecular nutrition, focusing on molecular and cell biology and investigating the molecular mechanism of adipose tissue differentiation. After that, I completed two postdoctoral projects focusing on human embryonic stem cell differentiation mechanisms and bioartificial liver development. I joined OXGENE in 2017, initially as a cell line engineering scientist. I then gradually started managing various functions within the company, including in cell sciences and biomanufacturing. Now, I head the department of engineering and production. Our department is responsible for the engineering and production of plasmids, viral vectors, and cell lines, aiming to provide high-quality starting materials for cell and gene therapy manufacturing.

Q In your own experience, what are the common pitfalls that therapy developers face when selecting the right plasmids for their application? What repercussions can making the wrong choice early on have later in development?

QL: From our interactions with customers in the past, we sometimes see that people fail to consider all aspects when they are selecting a plasmid. It can be easy

to miss things like regulatory compliance and intellectual property (IP) checks.

One example is some customers we had using special AAV vectors. They did not complete the IP checks in the early phase of the project and had to do so later on. It luckily did not result in any consequences in that case, but we might not be this lucky every time. If the IP side is not checked in the beginning, it might catch up with you in a later phase.

"One of the biggest challenges facing AAV gene therapy at the moment is the human immune system i.e. pre-existing immunity to the viral capsid."
- Richard Parker-Manuel, PhD

In terms of regulatory compliance, we also heard about a therapeutic company who did AAV production using a helper plasmid containing adenovirus late genes, which we believe are not recommended by the FDA. The company found this in the later stage of their product development process, which meant they would need to source a different helper plasmid and restart the program from early phase, or to do more work to justify their use of the helper plasmid containing the adenovirus late genes. These kinds of issues consume both extra cost and time.

RP: It all depends on the chosen vector system, because AAV vectors have different properties from lentiviral vectors. AAV issues could include choosing the wrong capsid for the target tissue, having a genome that is too large to fit in the capsid, or getting a high proportion of empty capsids, which is something that our modified Rep-Cap plasmid can help to avoid. One of the biggest challenges facing AAV gene therapy at the moment is the human immune system – i.e. pre-existing immunity to the viral capsid. One way to solve this could be by engineering the capsid proteins to avoid recognition by neutralizing antibodies. With lentiviral vectors, there is no structural capsid, so packaging size is slightly less of a constraint – although large genes can impact titers. Other transgene properties such as the presence of transcription termination signals can also cause low yields without careful optimization. It is important to design vectors with safety in mind from the outset, to avoid having to reconfigure them later. For instance, the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) in lentiviral vectors is often used, but the unmodified wild-type version can potentially cause tumorigenesis.

Q What are the important aspects developers should consider when selecting plasmids for their application, and what is your advice to developers looking to choose the right tools?

RP: The first consideration is which vector system to use. The plasmids are simply instructions for producing viral vectors, so it is important to have a clear idea about vector design early on. For AAV, this includes considerations such as which capsid to use, the

transgene structure, and whether to choose a single-stranded or self-complementary genome. The plasmids can then be tailored accordingly. Questions to ask include: have the vectors been designed with safety in mind? And does the developer have the freedom to operate with all the elements of the proposed vector design? In terms of productivity and potency, our modified Rep-Cap plasmid gives higher yields of full capsid AAV compared with the conventional configuration.

Q How early in therapeutic development should plasmid design and engineering be considered?

RP: Plasmid design and engineering should be considered as early as possible, during the discovery or pilot testing phases once the transgene has been chosen. Then, other elements such as promoter, polyadenylation signal, and plasmid backbones can be optimized. This way, early efficacy studies can use a vector that is as close to the final configuration as possible. Switching to a different design later could mean revalidating the new design, which could be costly and cause delays.

Q How can plasmid design help to address some of the current key challenges in cell and gene therapy development?

RP: The design of the genome plasmid can affect, not only yield, but also the quality of the viral vector that can be produced. For example, removing extraneous sequences from within a viral genome or adding stuffer DNA to the backbone could help improve the full/empty ratio of an AAV product.

The design of the trans-packaging plasmids is also important. For example, we found our lentiviral packaging plasmids outperformed those of competitors in head-to-head studies.

QL: From the safety side, we need to eliminate or modify potentially toxic genes or genes that could cause safety concerns, like wild-type WPRE sequences in lentiviral vectors or adenovirus late genes in helper plasmids for AAV production. This should be considered in the plasmid design phase. Another safety-related issue is replication competent virus generation, which is always something regulatory authorities pay attention to. In our plasmid design we try to reduce the homology of different viral packaging elements, and also try to separate different elements into different plasmids. This reduces the chance of homologous recombination, thus reducing the likelihood of generating replication competent viruses.

Q What are the benefits of working with a partner organization for discovery work, and are there particular benefits to partnering with a single CDMO across the whole therapeutic pipeline?

DOI: 10.18609/cgti.2022.140

QL: Reaching out to a contract development manufacturing organization (CDMO) for discovery work sounds a bit unusual – normally you might go to a contract research organization (CRO) to provide this kind of expertise. However, working with a CDMO that also has the capability and experience of doing discovery work, like OXGENE and WuXi Advanced Therapies, definitely provides an advantage. We can quickly figure out good approaches to achieve the clients' goals, and provide powerful platforms for designing and screening to make the process more efficient. For example, we have bioinformatics tools already in place, and high-throughput or automated platforms for screening purposes.

"CMDOs have extra advantages...particularly in process development and manufacturing capabilities" - Qian Liu, PhD

In addition, CDMOs can have extra advantages compared to CROs; particularly in process development and manufacturing capabilities. For example, when designing a discovery strategy, we will naturally consider the needs of subsequent stages, such as designing the products in an easier way for process development, scaling up, and manufacturing, whilst also considering regulatory compliance. This will reduce the risk of having to modify the design pathways throughout the journey. In other words, doing discovery work with experienced CDMOs can help you to see the bigger picture, as they will consider the whole journey for the customer. The product development lifecycle has many stages: discovery, research, development, manufacturing, and regulatory submissions. In all these stages, therapeutic companies need different services. If they had to go to different organizations to receive each service, they would have to communicate with many organizations. The time cost of communication can be huge. The end-to-end platform provided by WuXi Advanced Therapies reduces the time and cost that a customer needs to spend on communication. It provides a more streamlined platform, that makes the development of the product from beginning to end much easier for the customer.

AFFILIATIONS

Richard Parker-Manuel, PhD
Group Leader for Plasmid Engineering & Production,
OXGENE

Qian Liu, PhD
Head of Engineering & Production,
OXGENE

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: *Jul 19 2022;* ***Revised manuscript received:*** *Aug 12 2022;* ***Publication date:*** *Sep 7 2022.*

OXGENE™

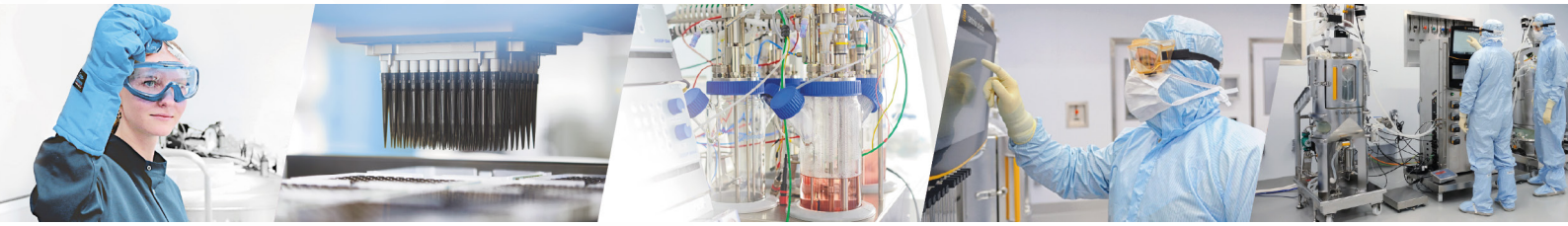
DOI: 10.18609/cgti.2022.140

WuXi ADVANCED THERAPIES 药明生基 | 药明康德 WuXi AppTec

High performance plasmids with exceptional lead times at all grades

We partner with innovators from discovery to commercialization.

- Plasmids available from research grade to clinical GMP
- Expert plasmid engineering and early discovery services
- Antibiotic-free GMP manufacture
- Delivered in two weeks



We manufacture and supply OXGENE's proprietary adeno-associated viral (AAV) and lentiviral plasmids royalty free. These plasmids are optimised to deliver high yields of high-quality viral vectors.

- Packaging plasmids available off the shelf for international delivery within 2 weeks
- Custom plasmids designed & delivered within 3 to 5 months (for research grade and commercial GMP respectively).
- Research grade plasmids manufactured and sold from the UK

Partner with us early in therapeutic development for accelerated timelines from discovery to commercialization

To view more, visit:
advancedtherapies.com

OXGENE®
A WuXi Advanced Therapies Company

FASTFACTS

Key benefits of a microfluidic platform for cell culture at a clinically relevant scale

James Kusena, Vice President Bioprocessing and Applications, MicrofluidX

Microfluidic cell cultures provide a range of advantages over traditional cell culture methods, including more precise measurement and control of the cell microenvironment, higher process yields and optimized reagent usage, as well as being a fully automatable and closed system. However, they have historically been applied almost exclusively at small scales.

A SCALABLE, AUTOMATED MICROFLUIDIC BIOREACTOR

The unique approach of the MicrofluidX bioreactor can utilize the benefits of microfluidic cell cultures for large quantities of cells (up to 100 billion), offering a scalable solution from process development to GMP manufacturing that does not require a change in cell environment **(Figure 1)**.

The MicrofluidX bioreactor is part of an automated cell therapy workstation for process development and manufacturing combining advanced end-to-end bioprocessing and analytical capabilities and scalability. This facilitates highly efficient bioprocessing of primary T cells, with five- to ten-times lower viral vector consumption, higher fold expansion, and tighter control of cell phenotype compared to conventional research cell culture vessels.

PRIMARY T CELL TRANSDUCTION

Five times greater transduction efficiency was achieved in primary T cell transduction using the MicrofluidX platform in comparison to the conventional well plate method, as shown in **Figure 2**. In addition, a higher level of homogeneity is possible, showing vector copy number distribution can be controlled, which is of clinical importance.

ADHERENT MSC BIOPROCESSING

Bioprocessing of adherent mesenchymal stem cells (MSCs) in monolayer in the MicrofluidX platform has been shown to surpass the performance of conventional cell culture vessels with a three-times higher cell density while maintaining cell phenotype, as illustrated by **Figure 3**. Cells expanded in the MicrofluidX bioreactor are also more compacted and confluent, while the viability remained similar: 92% in the well plate and 97% in the MicrofluidX platform.

Longitudinal studies over six passages of MSCs have shown 50% higher average and 30% lower variability in cell densities compared to conventional flask culture while maintaining cell phenotype across all passages.

Figure 1. Scalable microfluidic bioreactor from process development to manufacturing.

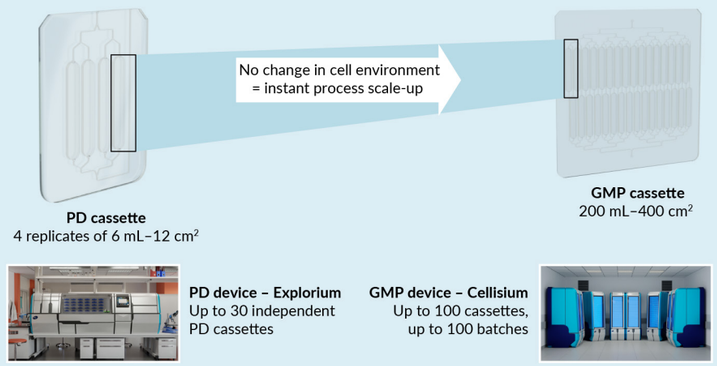


Figure 2. Primary T cell transduction with GFP lentiviral vector in MicrofluidX bioreactor versus well plate.

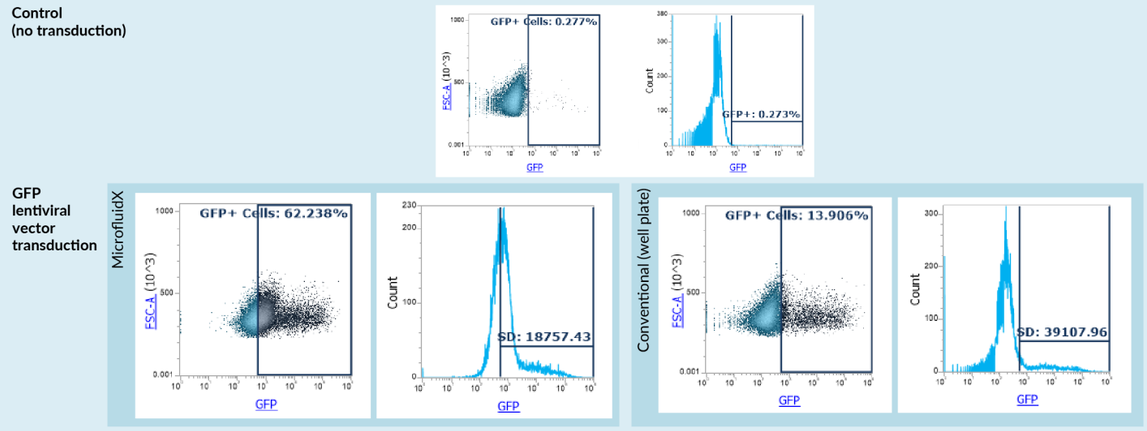
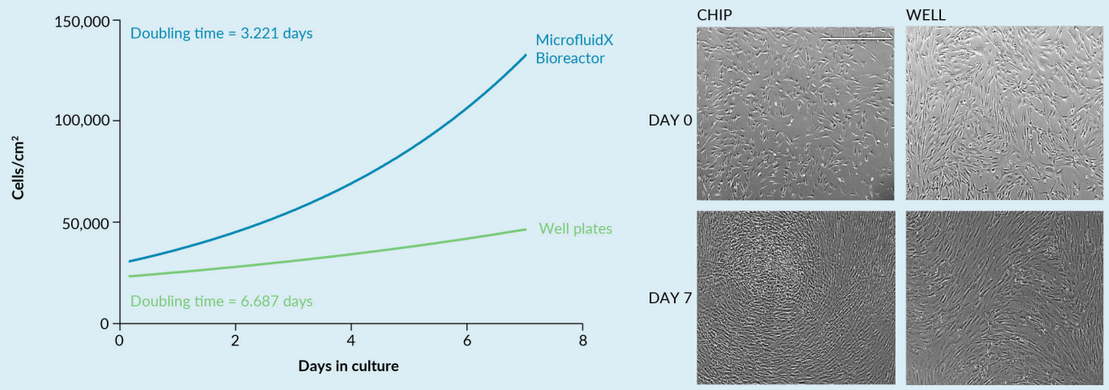


Figure 3. MSC growth curves in MicrofluidX bioreactor versus well plate after 7-day expansion in static culture.



CELL & GENE THERAPY INSIGHTS

Cell & Gene Therapy Insights 2022; 8(8), 1021; DOI: 10.18609/cgti.2022.151

In partnership with: microfluidx

FASTFACTS

Tailor your cell culture platform to achieve your research objectives & scale requirements

Ann Rossi Bilodeau, Senior Bioprocess Applications Scientist, Corning Life Sciences

The cell and gene therapy field is rapidly advancing, placing a focus on manufacturing challenges to meet the growing demand. The efficient harvest of viable cells or cell products and maintenance of native biological function influences platform and workflow choices. Established protocols for viral vector production on a range of scalable adherent platforms can be tailored to meet end objectives, taking into consideration factors such as desired yield, time to optimize the process, facility space, and media usage. Essentially, there is no 'one-size-fits-all' platform for upstream adherent cell culture.

SOLUTIONS FOR UPSTREAM ADHERENT CELL CULTURE

Corning offers manufacturing-ready platform solutions for upstream adherent cell culture allowing use for a diverse range of applications **(Table 1)**. The platforms each offer different advantages and disadvantages, meaning they should be matched to your research or manufacturing objectives.

Planar vessels such as Corning® CellSTACK® chambers, HYPERStack® vessels, and CellCube® modules are scalable, modular platforms that can be easily integrated into modular production setups, with available automation. In addition, the Corning Ascent® FBR (Fixed Bed Reactor) System is a closed-circulation system with process control designed to provide high-yield, and viable cell recovery. The process development scale system available now is scalable from 1–5 m^2. Pilot and Production scale Ascent FBR systems in development will scale from 20–1,000 m^2 for large-scale production. Microcarriers have multiple surface options, including Corning CellBIND® surface treatment, to tailor the surface to the specific cell type and application. When cultured in a bioreactor, the process control and scalability of a suspension platform can be achieved.

Table 1. Adherent culture platform comparison

Platform	Growth area (cm^2)	Medium volume (mL/cm^2)	Cell visualization	Required equipment
Polystyrene microcarriers	Variable	≥0.05	Depends	Spinner flask, magnetic stirrer or bioreactor, controller
Dissolvable microcarriers	Variable	≥0.05	Depends	Spinner flask, magnetic stirrer or bioreactor, controller
Corning CellSTACK Vessels	636, 1272, 3180, 6360, 25440	0.2–0.3	Depends (2 layers)	Incubators
Corning HYPERFlask Vessels	1720	0.326	4 to 10 layers	Incubators
Corning HYPERStack Vessels	6000, 18000	0.217	Depends (2 layers)	Incubators
Corning CellCube Modules	8500, 21250, 85000	0.1	Depends (2 layers)	Controller, oxygenator, warm room/heat blanket
Corning Ascent Fixed Bed Reactors	10000, 25000, 50000	0.11	Depends	Corning Ascent FBR System

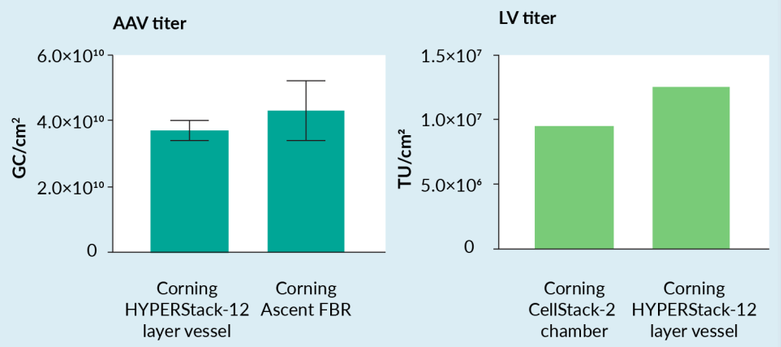
SCALE-UP OF MSCS WITH HIGH VIABILITY, MULTIPOTENCY MARKERS

Mesenchymal stem cells (MSCs) from several sources were expanded on the Corning CellBIND CellCube 25-layer module and the HYPERSTACK 36-layer vessel **(Table 2).** Human bone marrow, adipose, and umbilical cord-derived MSCs exhibit high viability, high density, cell expansion on the Corning CellBIND surface across multiple planar vessels with comparable yields on each platform tested. MSCs retain >99% expression of CD90/CD105/CD7, <0.5% hematopoietic lineage markers.

Table 2. Stem cell yields for Corning CellBIND CellCube 25-layer module and CellBIND HYPERStack 36-layer vessel.

Vessel	Cell type	Yield ($cells/cm^2$)
Corning CellBIND CellCube 25-layer modules	hBM-MSC	4.2×10^4
Corning CellBIND HYPERStack 36-layer vessel	hBM-MSC	4.4×10^4- 5.2×10^4
	hAD-MSC	2.7×10^4- 3.2×10^4
	hUC-MSC	6.1×10^4- 8.0×10^4

Figure 1. AAV titers for Corning HYPERStack 12-layer vessel and Ascent FBR and LV titers for Corning CellSTACK two-chamber and Corning HYPERStack 12-layer vessel.



SCALABLE VIRAL VECTOR PRODUCTION BY TRANSIENT TRANSFECTION

Vector yield from transient production systems can be improved with process optimization and transfection design of experiment (DoE).

Comparable productivity of adeno-associated virus (AAV) (GC/cm^2) was found on both the traditional planar Corning HYPERStack 12-layer vessel and the Corning Ascent FBR system **(Figure 1)**. Either platform could be used as a suitable method for AAV vector production, dependent on customer needs.

Higher infectious lentiviral (LV) titer yield (TU/cm^2) was achieved with Corning HYPER technology as the unique, gas-permeable surface provides favorable conditions for cell growth and LV packaging.

CONCLUSION

Choosing an adherent cell culture platform is a complex balance between several factors, including experimental objectives and desired scale.

CELL & GENE THERAPY INSIGHTS

Cell & Gene Therapy Insights 2022; 8(8), 1243; DOI: 10.18609/cgti.2022.181

In partnership with: CORNING

CELL & GENE THERAPY INSIGHTS

CELL & GENE THERAPY MANUFACTURING SCALE-UP/SCALE-OUT

SPOTLIGHT

INTERVIEW

Mentoring new talent in the cell and gene therapy manufacturing sphere

David McCall, Commissioning Editor, *Cell & Gene Therapy Insights,* talks to **Angela Collura**, Senior GMP Process Development Scientist, Achilles Therapeutics

ANGELA COLLURA completed her Post Graduation in Medical Biotechnology from Bologna University, in Italy. Angela went to work in Regulatory Affairs. After that she moved to the UK and joined the core GMP team at UCL Great Ormond Street Hospital where she was involved in the operations of the cleanrooms and the manufacture of immunotherapies such as CD19 CAR-T cells for Acute Lymphoblastic Leukemia (ALL) patients in a phase I/II clinical trial. Also, she had the opportunity to work in a trial where they manufactured an ATMP that was CD25/71 allodepleted T cells in patients undergoing hematopoietic stem cells transplant. Alongside the manufacture she was involved in the patients' immune-monitoring program. Next, Angela moved to the ATMP manufacturing and development platform at Guy's and St. Thomas' hospital. Here she worked as a Production Scientist first and then Senior Production Scientist in multiple clinical trial ATMPs ranging from T-Regs to Monocytes they developed and routinely manufactured for. Currently Angela works at Achilles Therapeutic where she is part of a team where they develop GMP compliant ATMP Production procedures for Achilles' pipeline products.

Cell & Gene Therapy Insights 2022; 8(8), 1015–1020

DOI: 10.18609/cgti.2022.150

Q What are you working on right now?

AC: I am currently working as a senior good manufacturing practice (GMP) process development scientist at Achilles Therapeutics. The focus of my role is to develop automated, closed, and robust manufacturing processes that will be translated to GMP.

At Achilles, we aim to develop personalized tumor-derived T cell therapy, targeting clonal cancer neoantigens. The current lead indications are advanced non-small cell lung cancer and recurrent metastatic melanoma – these are the subject of our CHIRON and THETIS clinical trials, respectively.

Both indications are characterized by their high mutational burden, high level of T cell infiltration, and high unmet medical need.

Q What are the key challenges facing cell therapy manufacturing from your perspective?

AC: Cell and gene therapy is a new branch of medicine that is revolutionizing the way certain diseases are treated, especially cancer. Chimeric antigen receptor (CAR T) cell therapies, which are a class of advanced therapy medicinal products (ATMPs), have changed the way we treat patients and CAR T has since become an important treatment modality for certain types of cancer such as B cell malignancies.

ATMPs are the first type of medicines that require scientists to work as part of a multidisciplinary team, consisting also of clinicians, nurses, clinical trial staff, and regulatory bodies. ATMP manufacturing presents challenges that require good understanding by all the parties mentioned above in delivering the treatment. It also requires organization, administration, and coordination with hospitals and university centers for successful planning and treatment of the patients. Therefore, scientists, regulators, and the healthcare workforce require a common training platform that can enhance communication and collaboration between all parties.

There is a need for awareness of the intricacies and challenges in production and design to be integrated into teaching programs. Some universities offer educational programs around ATMPs, but most of them are not currently integrated at the earlier stages of education.

Traditionally scientists are trained in research laboratories, often in universities, which is in contrast with the strictly regulated working environment of companies developing and manufacturing ATMPs. The manufacture of these products often requires

> "The field is evolving continuously and quickly. It presents many gaps that need to be filled by multiskilled talent. Those gaps represent a great opportunity for the junior members of staff to fit in with a diverse set of technical and soft skills."

DOI: 10.18609/cgti.2022.150

long cleanroom hours, weekend work and strict adherence to documented process and procedures. These good documentation practices can also initially seem tedious if one is trained in traditional research laboratories. Therefore, hiring and retaining staff who are willing to work in this environment is challenging and requires good training plans to be in place to assure required competency levels can be achieved and standardized.

Q Do you believe that young people entering the cell and gene therapy space today generally feel that they have a clear career pathway in front of them?

AC: The field is evolving continuously and quickly. It presents many gaps that need to be filled by multiskilled talent. Those gaps represent a great opportunity for the junior members of staff to fit in with a diverse set of technical and soft skills.

The training offered by many organizations such as the International Society of Cell and Gene Therapy (ISCT), and private companies, is growing exponentially. Mentorship programs can also be a good opportunity for future career progression and development in numerous directions.

Direct experience on the ground can give each worker exposure to many opportunities, enabling them to discover where their ambition and skills lie. There can sometimes be an ambiguity around potential career pathways within the field, but it can be tailored to each individual depending on their technical and soft skills.

Q Why is it so important for new workers to gain a broad understanding of the field – for instance, the importance and impact of the regulatory environment? What are the current barriers here?

AC: ATMPs are a unique set of biological drugs which require some degree of understanding of the various stages of drug development. The journey of ATMPs starts in a research laboratory, gets refined in a process development lab and is then tech transferred into the GMP environment. The manufactured drug product then goes to a clinical center where it is infused into the patient at their bedside. Therefore, each of these stages has its own complexities and challenges that need appreciation and some degree of understanding by staff working at each different stage of development. However, it is certainly useful and helpful for all staff to have a broad understanding of each of the stages in order for the product to be successfully delivered to a patient.

The production of ATMPs must be compliant with GMP, therefore job roles in GMP production, for example, are multidisciplinary roles. Not only do they require knowledge of immunology, cell biology, and aseptic technique, but also a good understanding of regulatory documents, and their interpretation.

This might not be immediately obvious for someone who is beginning their work in this field. A current barrier for junior members of manufacturing staff is understanding the

regulatory aspect of the job, which is usually confined to the quality or regulatory departments. It may be beneficial to organize interactive courses held by the quality and regulatory departments to give everyone a broad overview of the regulatory area. Furthermore, current teaching programs should include education around regulations concerning ATMPs.

Q What are the obstacles to multiskilling new staff, particularly in terms of soft skills?

AC: Currently, junior members of staff are trained mainly in technical and regulatory aspects. There is not enough investment in training in soft skills such as communication, teamwork, problem-solving, understanding how to de-escalate difficult situations, and being able to work with different types of personalities on the ground.

Usually, these are characteristics that hiring managers look for in people, but there is not enough training done once a new starter is beginning their job. Once a candidate is hired, HR can help identify who may benefit particularly from soft skills workshops.

Communication between senior management staff should exist more as a mentoring relationship, where case scenarios can be presented from their past experiences. An open dialogue between staff should be encouraged (for example, between quality and production) by ensuring the training of junior staff members is efficient to the point where they are empowered to fill the gaps in the field with their own talents. These talents can vary greatly, encompassing things like organizational, training, interpersonal skills, and motivation for producing potentially game-changing engineered cell therapies for patients.

Q Can you expand upon the role that mentoring should play in addressing these issues?

AC: In my career, I have been lucky enough to come across many passionate, knowledgeable, and experienced lead scientists and managers, who perform their jobs with integrity and engagement. They are aware that the ultimate goal of this strictly regulated environment is patient safety and health. They have taught me many things, but the most important lesson is that our job is unique and relatively new, and therefore requires enthusiasm and a pioneering mentality.

Some universities have interdepartmental mentorship schemes in place. Creating mentorship programs within the private sector would be beneficial to create connections between differently skilled workers. Mentorship programs might be the way to transfer those soft skills and knowledge to inspire the younger generations to join the ATMP workforce.

"Currently, junior members of staff are trained mainly in technical and regulatory aspects. There is not enough investment in training in soft skills..."

DOI: 10.18609/cgti.2022.150

Again, from my experience, training is sometimes only given in technical skills, but it is important for senior members of staff to sit down with juniors and transmit both experiences and also a passion for the job. Each product in cell and gene therapy is different, and you must take into consideration that there is a patient at the end of the product life cycle.

Q What misconceptions might new entrants into the cell and gene therapy workforce have, and how can ongoing training be adapted to address them?

AC: Common misconceptions relating to production scientists include people often believing that the role is flexible. They associate the word 'scientist' with the ability to be flexible and creative, and to freely change how you do things during GMP production. Of course, this is not the case in reality, because we need to strictly adhere to the established manufacturing procedures.

People may also think that it is a '9-to-5' job, and that is not true either. Some junior members may think that being a production scientist only means being an operator who follows instructions without thinking or having holistic scientific knowledge. There is a structured way of de-escalating issues or conflicts, but you need to know what to do in every situation – it is so important to have a broader understanding of cell therapy drug development process. Further, it is important to understand that manufacture of the ATMPs should ensure that the product quality is 'fit for its intended use'.

Training by experienced and knowledgeable staff members is the priority. For example, before someone can use the cleanroom to produce an ATMP, it is important to conduct qualification exams to assess their understanding of the product. Senior staff members should present case scenarios and explain the troubleshooting process to overcome any issues that can occur during the manufacture of the product.

Q Can you sum up some key goals and priorities, both for yourself in your role and for Achilles Therapeutics as a whole, over the next 12–24 months?

AC: My ultimate goal is to transfer new processes from process development to the GMP environment, ensuring compliance with the critical quality attributes and critical process parameters of these ATMPs by following the Quality by Design principle.

This is to ensure consistency of process among different patients. Achilles is striving to find a personalized treatment for those patients who currently do not have long term successful treatment options.

The way for Achilles to achieve its goal is through precision medicine and the use PELEUS™, our proprietary AI-powered bioinformatics platform. Achilles is currently analyzing clonal mutations for each patient, and then producing a cell therapy with the ability to target their tumor cells without damaging their healthy tissues.

AFFILIATION

Angela Collura
Senior GMP Process Development Scientist,
Achilles Therapeutics

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 based on an interview with Angela Collura carried out on Jul 4 2022.*

Interview held: *Jul 4 2022;* ***Revised manuscript received:*** *Aug 31 2022;* ***Publication date:***

DOI: 10.18609/cgti.2022.150

SEPTEMBER 2022 Volume 8, Issue 8

CELL & GENE THERAPY INSIGHTS

LATEST ARTICLES:

INTERVIEW

Learning from pediatric CAR-T development: insights from manufacturing unique patient doses

Róisin McGuigan, Editor, *BioInsights,* speaks to **Chris Brown,** Director, GMP Production, Seattle Children's Hospital and **Sean Werner**, CTO Cell Processing, BioLife Solutions

CHRIS BROWN is the Director of GMP Manufacturing within the Therapeutic Cell Production Core, Seattle Children's Therapeutics' GMP cell manufacturing facility. He leads the facility's manufacturing/process development team and played a key role in initial design, stand-up, and ongoing development of the TCPC facilities, team, and manufacturing methodologies. He has more than 20 years' experience in the manufacturing of cellular products for Phase 1 and 2 clinical trials, with a focus in translating cutting-edge research into first-in-human cellular therapeutics. He joined Seattle Children's Research Institute from the Fred Hutchinson Cancer Research Center in 2010, where he led the manufacturing team within the Cellular Production Facility cleanroom. He holds a BA in Biology from Carleton College in Northfield, Minnesota.

SEAN WERNER is the Chief Technology Officer – Cell Processing at BioLife Solutions, a leading provider of bioproduction tools and services to the cell and gene therapy and broader biopharma markets. BioLife acquired Sexton Biotechnologies in 2021 where Sean was President of the company known for providing processing and handling solutions for the CGT industry. Sean received his PhD from Purdue University in Biology followed by post-doctoral positions at the Indiana University School of Medicine and Eli Lilly. Sean has previous experience filling various roles in the global regulatory and general management functions supporting medical devices, autologous cell therapy, and single use disposable development programs. In his 15 years working in the life science industry, he has guided pre-clinical and clinical testing and submission strategies leading to global commercialization of multiple medical devices and bioprocessing tools.

Cell & Gene Therapy Insights 2022; 8(8), 1071–1078
DOI: 10.18609/cgti.2022.158

Manufacturing for pediatric indications provides important lessons to broader cell and gene therapy manufacturing. As the applications of cell therapy expand in adult cancers and solid tumors, it is important not to forget the lessons learned from first-generation cell therapies. In this episode, Sean Werner and Chris Brown discuss the unique perspectives those working on pediatric therapies can offer to help move the industry forward.

Q What key lessons has the cell and gene therapy industry learned from pediatric therapies that can be applied to developing cell therapies for other indications, such as adult cancers and solid tumors?

CB: One of the real drivers that we have learned from pediatric therapies is the requirement to do what we can with a relatively small starting number of cells. Work on pediatric patients often involves smaller apheresis products, or in some cases peripheral blood as the starting material. This limits the size of the culture that one can target, and the up-front manipulations which may or may not be possible or necessary. Being able to manufacture a product with a smaller starting material is a benefit for all sorts of trials, in terms of the number of shots on goal you might have in the event of a manufacturing failure.

SW: It is really interesting to consider the limited starting material as a key element of that and thinking through what we as an overall industry hope to be coming to: larger scale manufacturing, moving from autologous into allogeneic therapies, and trying to understand how people working on these pediatric therapies have overcome the limitations and applying that to make sure that something is actually manufacturable once you're targeting those other indications.

Q When it comes to the development of cell therapies for these indications, what for you represents the cutting edge in terms of tools and technology? Where is improvement or innovation most needed to meet both immediate and longer-term needs?

SW: If you think of cell therapy as the next step in the development of the overall pharmaceutical industry, I think what's really the cutting edge is for us to move more towards what large molecule pharma has – closed systems, and integration up and down the chain in terms of the unit operations. The industry largely developed out

of academic work and individual tools that were borrowed from other industries and other places, and now we're at the point where what's really going to advance us forwards is the ability to have these fluid, streamlined manufacturing processes. Whether that is at a good manufacturing practice (GMP) center or at a commercial enterprise, while there will be differences between the two, in the end being able to do highly qualified GMP manufacturing reproducibly, no matter what the scale, is really the cutting edge.

> "One of the real drivers that we have learned from pediatric therapies is the requirement to do what we can with a relatively small starting number of cells."
> - Chris Brown

CB: I strongly agree that closed system manufacturing is where we need to go. Obviously, I operate in more of an academic setting, and one can absolutely bring a Phase 1 and sometimes a Phase 2 trial forward with very traditional, open-system manufacture. But that just kicks the can down the road in terms of all of the qualification-type work that you have to do anyway prior to taking it into Phase 3 or further manufacture. A focus on that closed-system manufacturing and optimization with an eye towards future commercialization from the very early stages is really important. It is something that we have focused on very heavily at Seattle Children's.

Another interesting area that comes from the pediatric space is that the older days of cell therapy often involved huge doses, large culture bags, and sometimes a billion cells or more. The development of the final product storage and administration vessel as an integrated vessel, thawing device and a tubing set, with an eye towards integrated thawing at the bedside of a much smaller number of cells, has been critical for our success and for the development of the systems that we are currently using in our manufacturing operations.

Q Turning to cost and funding, what would you identify as the biggest challenges in this area?

CB: One of the most important challenges regarding cost and funding, specifically from the pediatric environment, is what percentage of that funding for cell therapy and oncology research in general actually comes to the pediatric space as opposed to the adult space. I don't have the exact percentage off the top of my head, but the last time I looked it was in the single digits. It requires a large reliance on philanthropy and non-traditional fundraising sources in order to maintain the manufacturing and research and development expertise required to keep pushing that cutting edge forward. That will likely always be a challenge, and certainly it is one that we and other people in the pediatric space continue to face.

SW: That is a really interesting point. I have some previous background in medical devices, and I know from the supplier side that it is a challenge to figure out everything needed when something is being developed explicitly for pediatrics. Firstly, the overall patient population may be very small, and secondly, the safety and efficacy expectations along with doing the clinical work can be very difficult. On the supplier side it is an interesting challenge to think about how to successfully develop something that is intended for out-of-the-gate pediatric use. I would love to spend some time thinking about how we can get better on that.

To add to that, from our conversations that we have with manufacturers and academic folks, they are a little different. However, one of the things that we hear a lot is that the facilities required to operate in the current state – using open processes and very manual things – are very high cost, very expensive to maintain and operate, along with making sure that they are up to the standards that are expected. Another aspect is people. We are operating in an environment where we have extremely highly trained folks doing these processes, and that's a big investment both in time and direct resources.

I think the component costs, the supplies and reagents, will moderate when we get to scale and get to where we as a supplier can anticipate what our cost of goods is going to be. I think that over time we can come to costs that will make sense. However, the people and the facilities are a long-running challenge that we're going to have to think about.

CB: I certainly agree that cost of goods is a very small overall portion of the cost to manufacture these products. To maintain a large facility and a very talented network of folks on the manufacturing, quality control, quality assurance, facilities, and operations side is much closer to biotech start-up costs than to academic research lab costs.

Figuring out how to fit that into what is often a not-for-profit model can be very challenging. These are expensive therapies to manufacture, and this is a necessary step in bringing them towards first-line therapies for kids throughout the world who don't have access to this kind of research.

Q From a supplier perspective, what would you say works well – or doesn't work well – when trying to approach and solve customer challenges?

SW: One thing that seems to be working well is the understanding at a high level of what processes our customers are carrying out. As a supplier we generally know the manufacturing step, sometimes in quite a lot of detail, so we can modify things that are already in our toolbox relatively quickly. The more communication and conversation we can have with our customers the easier it is for us to help develop solutions. This may be either figuring out the right workflow to use existing tools, or in some cases realizing that there's a specific need to develop a modification of a tool, or a brand-new solution.

One of the things that is harder to address, and maybe isn't working well, is considering where we are going to be in five years, or ten years. For some of these tools the development cycle is eighteen to twenty-four months. For that to be aligned with when people need

DOI: 10.18609/cgti.2022.158

it, earlier conversation about where they are going is going to be helpful. The more conversations we have with our customers, the easier it is for us to help solve their problems. Getting involved in what may be coming downstream is going to be important for us in order to align on when scale-out and scale-up processes are actually needed.

> "We need recognition on both sides that there are going to be stumbles and there are going to be wins, and we're all in it together."
> - Sean Werner

CB: I want to echo the idea of frequent and open communication between users and suppliers. I would add that often, especially in these early phase trials, we find ourselves in unexpected situations. Maybe we're using new equipment or new supplies. When something performs in a way that we don't expect, when we have a patient starting material that doesn't expand in the way we want, or on the rare occasions when we have an issue with a supply, it is critical to have very open and bidirectional communication between the user and supplier. This helps to get to root causes, put containment measures in place, and if necessary to make longer-term changes either to the process or to the product to avoid recurrence.

There is a great deal of expertise both on the user and on the suppliers' side, and close and open communication is what makes that a positive learning experience, allowing you to go from unexpected outcomes to developing a better future state of product.

SW: It is really nice to hear that validation of the idea of trying to be open. A lot of people are pretty closed in our experience. But if we don't know what is or isn't working on the floor, it is really hard for us to be responsive.

The other piece of it is that we are growing along with the developers, and the idea of manufacturing tools specifically for cell and gene therapies is no older than the cell and gene therapy industry itself. As our customers are learning and growing and figuring out how to be successful, and as GMP centers are expanding the horizon of what they're taking on in terms of clinical work, that's where we are too.

It's important to recognize that most of us anticipate changes will be needed, and we all have the same downstream vision of treating these patients. We need recognition on both sides that there are going to be stumbles and there are going to be wins, and we're all in it together.

Q What are the biggest lessons gleaned from first generation cell therapies that can be carried forward when defining and developing state-of-the-art cell therapy manufacturing approaches?

CB: I think back to the trials that I've participated in at the beginning of my career in manufacturing, around the year 2000. I have distinct recollections of six, eight, or ten-liter culture bag harvests, or even of 200 T-flask harvests, and of pouring the T-flasks into conical tubes for open-system spinning. We would generate huge numbers of cells that we would be administering to patients, and somewhere in that giant cell population were the specific cells that are going to make a difference *in vivo*.

So from my perspective, and in terms of the scaling of the manufacturing processes to treat more patients, better identifying the specific cell type that we are looking for and expecting to make a difference is really important. We want to optimize the manufacturing process and move from generating a giant bulk suspension to generating a much smaller number of highly defined cells that we expect to make a difference *in vivo*. We want to cut the manufacturing time down from months of repeated stim cycles, to a short-term culture method where we have cell product ready for patients much quicker. This will reduce the overall vein-to-vein time, and give us a system that can be scaled.

If we are not spending three months manufacturing a product, but instead spending seven days, that's significantly more patients that we can treat with the same facilities, and with the same staff requirements. The aim is turning this from a more boutique, artisanal manufacturing process to something that can be scaled; something that would be amenable to situations much more like simple A-line manufacturing.

SW: I will build directly from Chris's discussion about identifying the important cell and making sure we are doing that right, to looking at the potency assays that are valuable for this.

Potency assays shouldn't necessarily predict a clinical outcome, but they should predict clinical function or biological function. In the last few years we've gone from marker-based quasi-potency to developing assays that are showing 'these are the intended cells and in this *in vitro* environment they should X, and I can consistently get that X.'

As we go forward, this is something that has been missing – figuring out early-on with new concepts what is going to be the important potency assay and then using that to define your boundary conditions and your parameters. If we need to make changes post-licensure, or if we need to make manufacturing modifications, we need to make sure that we can actually create a product every time we go through. If you don't know what those boundary conditions are, you can't make that change very easily. And if you don't have the right potency assay developed early on, you can't define what those boundary conditions are. The future is just continuing on that step – now that we can say we have the right cells, we need to be able to say 'and they're doing the right thing.'

CB: That says it really well. I would add in the importance of being able to understand what would be predictive of manufacturing failure specifically, in terms of developing a manufacturing process that is relatively tolerant for the unusual and often very different conditions that we would see in patient material from different disease states. And even within the same disease state with individual person-to-person variability. We are not making widgets quite yet. But in order to scale these processes, we eventually need to have a system that is much more like making widgets, with the same outcome every time.

DOI: 10.18609/cgti.2022.158

AFFILIATIONS

Chris Brown
Director GMP Production, Seattle Children's Hospital

Sean Werner
CTO Cell Processing, BioLife Solutions

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: *Sean Werner discloses he is a full time employee of Sexton Biotechologies and BioLife Solutions The authors have no other conflicts of interest.*

Funding declaration: *Sean Werner received financial support for the research, authorship and/or publication of this article from Sexton Biotechologies and BioLife Solutions.*

ARTICLE & COPYRIGHT INFORMATION

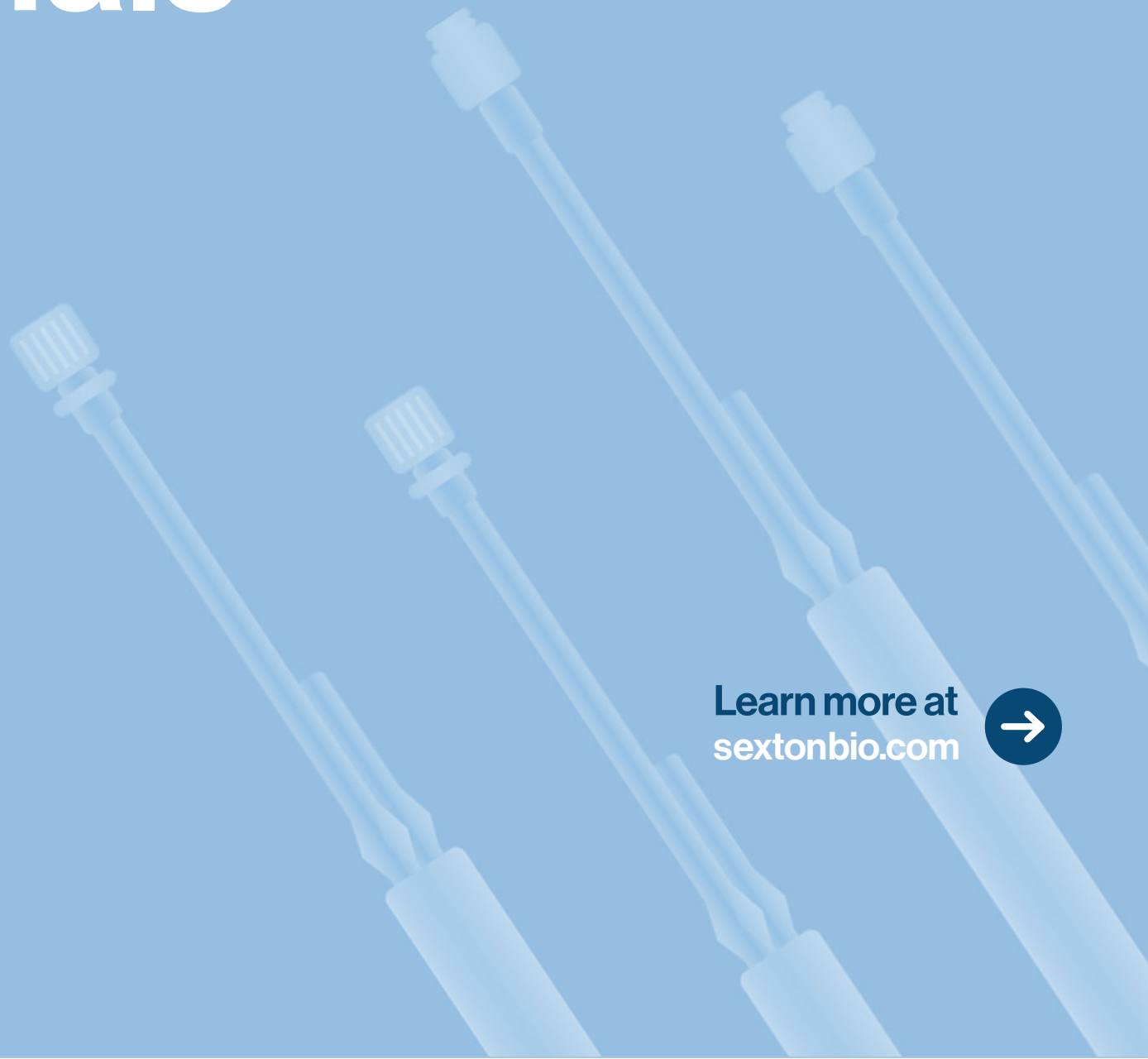
Article source: *This article is based on an podcast interview with Chris Brown and Sean Werner carried out on Sep 6 2022.*

Interview held: *Sep 6 2022*; ***Revised manuscript received:*** *Sep 14 2022*; ***Publication date:*** *Sep 05 2022.*

Sexton Biotechnologies®

Part of Biolife Solutions®

CellSeal Cryogenic Vials



Learn more at sextonbio.com

CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Evaluating DNA purity ratio determination with the CTech™ SoloVPE system®

Nigel Herbert, Drusha Purohit & Hannah Mignault

Spectrophotometric analysis is one of the most common techniques used to quantitate nucleic acids in a solution. More specifically, the 260/280 UV absorbance ratio of the nucleic acid can be used to determine their purity [1]. However, traditional fixed-pathlength spectrophotometers have limitations when determining the purity ratios of these molecules. In this study, the CTech™ SoloVPE® system assessed theoretical DNA purity ratios by utilizing its variable pathlength method, known as Slope Spectroscopy®. The method was evaluated by assessing the specificity, intermediate precision, repeatability, linearity, and accuracy of the theoretical purity ratios. The observed purity ratios from the SoloVPE system demonstrated great comparison to the theoretical purity ratios, verifying the SoloVPE system's slope spectroscopy method to be preferable for this application.

Cell & Gene Therapy Insights 2022; 8(8), 1047–1054

DOI: 10.18609/cgti.2022.155

INTRODUCTION

Gene therapy is an emerging medical approach to treating, preventing, and curing a wide range of genetic diseases. By administering genetic material into defective cells, the transgene has the potential to repair or enhance the cell's native genetic material. In order to successfully implement this approach in downstream bioprocessing, it is imperative to understand the purity of DNA. The purity is related to the quality and in many instances, the efficacy of the samples, ensuring that they are free of proteins, lipids, salts, and other contaminants [2].

UV-Vis spectroscopy is the most common analytical method to determine DNA purity. The ratio of the absorbance at 260 nm and 280 nm is used to analyze the purity of nucleic acids [3]. This ratio is widely known as the R value, where pure DNA260/280 is between 1.8 and 2.0 [3]. Since proteins absorb at 280

nm, this ratio is used to assess the amount of protein contamination within the sample [4]. Impurities in DNA can lead to inaccurate measurement of DNA concentration and risk adversely impacting any subsequent downstream processes or therapeutic potential [5].

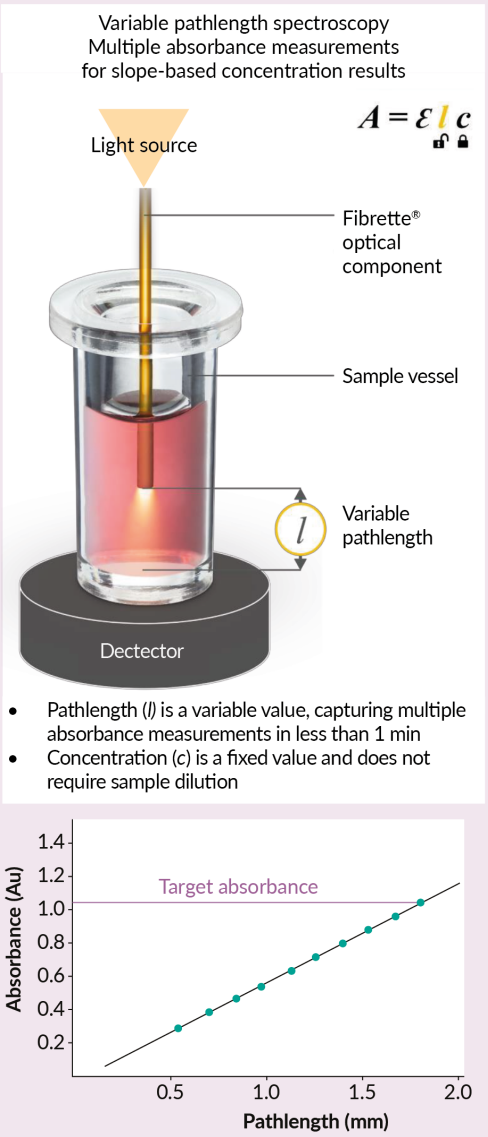
Traditional UV-Vis spectrophotometers utilize the Beer-Lambert law to calculate a sample's concentration. Beer's law states that A = ε*l*c where A is the measured absorbance, ε is the molar absorption coefficient, l is the pathlength, and c is the concentration of the sample. Since traditional spectrophotometers utilize a fixed 1 cm pathlength, samples may require dilution which can lead to assay error. In the case of highly concentration DNA, samples must be diluted to ensure that the assays are within the linear range of the instrument. Because of the instrument's limit of detection, linear range, and fixed pathlength, serial dilution is required, which can introduce error.

The SoloVPE System is an increasingly popular UV-Vis technology that utilizes the Slope Spectroscopy method to analyze sample concentrations. The Slope Spectroscopy method is an analytical manipulation of Beer's law that allows the SoloVPE System to perform variable pathlength measurements. Rather than relying on a single absorbance value, the Slope Spectroscopy method creates section data based on the collected absorbance values per pathlength. To enable the Slope Spectroscopy equation, the pathlength term l is moved to the left side of the equation where A/l = ε*c. The A/l term is the change in absorbance per change in pathlength, which is also known as the slope m of the equation. This substitution results in the Slope Spectroscopy equation which can be expressed as m = ε*c. The slope is the most critical value within the equation, as it allows us to determine the sample concentration or molar absorption coefficient [6].

The SoloVPE System defines its pathlength range by measuring the distance between the bottom of the CTechTM Fibrette® Optical Component and the bottom of the sample vessel. The SoloVPE System's integrated hardware and software allow it to move the Fibrette Optical Component up and down from 5 µm to 15 mm, with a pathlength resolution of 5 µm steps (Figure 1). The linear regression coefficient (R2) of the measurement confirms the correlation with Beer's law. The

FIGURE 1

Mechanism of variable pathlength UV-visible technology (VPT).



l: The distance between the tip of the light-delivering Fibrette Optical Component and the inside bottom of the sample vessel.
In this publication, the SoloVPE System demonstrates why the Slope Spectroscopy method is the optimal technique when analyzing nucleic acid purity required for therapeutic delivery systems or raw material in gene therapy applications.

DOI: 10.18609/cgti.2022.155

SoloVPE System requires all measurements have an R2 ≥ 0.999 to be considered valid. Values close to one confirm a strong correlation with Beer's law by demonstrating that the absorbance values change proportionally with the pathlength values. Therefore, the SoloVPE System can measure wide ranges of concentration without the need of extensive sample preparation and dilution. The SoloVPE System's variable pathlength technology, fast analysis speed, and enhanced spectral range allow it to produce accurate, linear, and repeatable results [6].

In this publication, the SoloVPE System demonstrates why the Slope Spectroscopy method is the optimal technique when analyzing nucleic acid purity required for therapeutic delivery systems or raw material in gene therapy applications.

MATERIALS & METHODS

Materials

The materials and consumables used to analyze the DNA purity ratios are listed below. Charles River provided DNA and protein, which were purchased from Thermo Fisher Scientific. The SoloVPE System and associated consumables were provided by Repligen.

Charles River Laboratories

- DNase/RNase free distilled water (Catalog No. 10977015 / Lot No. 2277167)
- Calf thymus DNA (Catalog No. 15633019 / Lot No. 2187506)
- Bovine serum albumin (Catalog No. 23209 / Lot No. WF329717)

Repligen

- SoloVPE instrument (Part No. SYS-VPE-SOLO5)
- Cary 60 UV-Vis spectrophotometer (Part No. IN-CARY 60 or Agilent Part No. G686OA)
- Fibrette Optical Component (Part No. OF0002-P50)
- Plastic vessel–small (Part No. OC0009-1-P50)
- Sample vessel holder–small (Part No. HM0178)

Methods

Theoretical DNA purity ratios were analyzed by formulating dilution levels of calf thymus DNA and bovine serum albumin with DNase/RNase-free distilled water. The theoretical purity ratios were calculated by using the average slope of BSA and DNA at 260 nm and 280 nm (from 100% protein and 100% DNA sample readings) and applying the following formula:

$$\text{Theoretical Purity} = (\%\text{Protein} * \text{Slope}_{\text{Protein@260}}) + (\%\ \text{DNA} * \text{Slope}_{\text{DNA@260}}) / (\%\text{Protein} * \text{Slope}_{\text{Protein@280}}) + (\%\text{DNA} * \text{Slope}_{\text{DNA@280}})$$

Eight purity levels were made, starting with 100% protein, and ending with 100% DNA (Table 1).

The SoloVPE System's small plastic sample vessel was used and required only 120 ul of sample volume. Data was collected in triplicate at each purity level with two analysts during different days.

RESULTS

Specificity

The specificity was assessed by determining if baseline correction was required for all analyses. The need for baseline correction was analyzed by measuring the buffer at 260 nm and 280 nm. Generally, baseline correction is not required if the slope of the buffer is < 0.01 Abs/mm. At slopes this low, the R^2 criterion of ≥ 0.999 is not required. This criterion demonstrates if there is any absorbance contribution from the buffer. In this study, the DNase/RNase-free distilled water was measured in triplicate. The average slopes at 260 nm and 280 nm were 0.00106 and 0.00094 Abs/mm respectively. Table 2 demonstrates that the measured slopes are well below 0.01

TABLE 1

Purity ratio levels.

Dilution level	% protein	% DNA	Total volume (µl)	BSA (µl)	DNA (µl)
1	100.0%	0.0%	750.0	750.0	0.0
2	85.0%	15.0%	750.0	638.0	112.5
3	70.0%	30.0%	750.0	525.0	225.0
4	50.0%	50.0%	750.0	375.0	375.0
5	35.0%	65.0%	750.0	263.0	488.0
6	10.0%	90.0%	750.0	75.0	675.0
7	5.0%	95.0%	750.0	37.5	713.0
8	0.0%	100.0%	750.0	0.0	750.0

TABLE 2

Observed slope values of the DNase/RNase-free distilled water. The data demonstrates negligible absorbance contribution from the buffer.

Slope (Abs/mm)	Average slope (Abs/mm)	Slope (Abs/mm)	Average slope (Abs/mm)
260nm		280nm	
0.00110 Abs/mm	0.00106 Abs/mm	0.00090 Abs/mm	0.00094 Abs/mm
0.00099 Abs/mm		0.00104 Abs/mm	
0.00108 Abs/mm		0.00080 Abs/mm	

Abs/mm, indicating that baseline correction is not required.

Intermediate precision

The intermediate precision was assessed by evaluating the % error of the average observed purity ratios against the theoretical purity ratios. Each purity level was measured in triplicate over the course of four days with two different analysts. For this study to pass, the % error must be ≤ 5.00%. Each triplicate reading demonstrated a % error less than the acceptance criteria of 5.00% as shown in Table 3. The neat protein (level one) and neat DNA (level eight) samples showed the greatest agreement between the expected and observed purity ratios. The results of the intermediate precision study demonstrate that the method is precise.

Repeatability

The repeatability of the system was assessed by evaluating the percent relative standard deviation (%RSD) of all the triplicate reads. The measurements were taken using the same sample, Fibrette Optical Component, and sample vessel. The %RSD is calculated by using the following formula: %RSD = (Standard deviation / average) * 100. For the triplicate reads to be considered repeatable, the %RSD must be ≤ ±2.00%. As shown in Table 3, the %RSD was well below ±2.00% for each triplicate reading.

Linearity

The linearity was assessed by analyzing the average R^2 of each triplicate reading (Table 4). The R^2 must be ≥ 0.999 for the measurement to be considered valid and linear. As demonstrated in Table 4, the average R^2 was well above 0.999 at each purity level during different days and with two analysts.

Additionally, the linear regression of the dilution series was analyzed by plotting the theoretical purity ratios against the average observed purity ratios. Figure 2 demonstrates each of the linear regressions. The analysis that was performed on day 1 with analyst 1

DOI: 10.18609/cgti.2022.155

TABLE 3

% Error of the theoretical purity ratios against the average observed purity ratios, and the %RSD for each triplicate measurement. The observed purity ratios correlate very well with the theoretical purity ratios, and the triplicate measurements demonstrate great precision.

Analyst & day	Dilution level	Theoretical purity ratios (Abs/mm)	Observed purity ratio #1 (Abs/mm)	Observed purity ratio #2 (Abs/mm)	Observed purity ratio #3 (Abs/mm)	Average observed purity ratio (Abs/mm)	Expected vs. observed %error #1 (%)	Expected vs. observed %error #2 (%)	Expected vs. observed %error #3 (%)	Expected vs. average observed %error (%)	Standard deviation	% RSD
Day 1, analyst 1	1	0.712	0.7112	0.713	0.7115	0.7119	0.14%	0.07%	0.01%	0.14%	0.001	0.14%
	2	1.6043	1.6117	1.6203	1.6213	1.6178	1.00%	1.06%	0.84%	1.00%	0.0053	0.33%
	3	1.7541	1.7625	1.8026	1.7756	1.7802	2.77%	1.22%	1.49%	2.77%	0.0205	1.15%
	4	1.8291	1.8571	1.8598	1.8558	1.8576	1.68%	1.46%	1.56%	1.68%	0.0021	0.11%
	5	1.8577	1.8908	1.8884	1.8972	1.8921	1.66%	2.13%	1.86%	1.66%	0.0046	0.24%
	6	1.8854	1.9226	1.9373	1.9318	1.9306	2.75%	2.46%	2.39%	2.75%	0.0074	0.38%
	7	1.8893	1.9184	1.915	1.9195	1.9177	1.36%	1.60%	1.50%	1.36%	0.0023	0.12%
	8	1.8929	1.883	1.8992	1.8965	1.8929	0.34%	0.19%	0.00%	0.34%	0.0087	0.46%
Day 2, analyst 1	1	0.7131	0.7145	0.7125	0.7128	0.7133	0.19%	0.08%	0.05%	0.02%	0.001	0.15%
	2	1.5971	1.5988	1.6013	1.594	1.598	0.11%	0.26%	0.20%	0.06%	0.0037	0.23%
	3	1.7516	1.7561	1.752	1.7537	1.7539	0.26%	0.02%	0.12%	0.13%	0.0021	0.12%
	4	1.8296	1.8287	1.8526	1.8446	1.842	0.05%	1.25%	0.82%	0.67%	0.0121	0.66%
	5	1.8595	1.8925	1.8959	1.8981	1.8955	1.78%	1.96%	2.08%	1.94%	0.0028	0.15%
	6	1.8885	1.9098	1.9229	1.9355	1.9227	1.13%	1.82%	2.49%	1.81%	0.0129	0.67%
	7	1.8926	1.9266	1.9305	1.9378	1.9316	1.79%	2.00%	2.39%	2.06%	0.0057	0.30%
	8	1.8963	1.8807	1.8889	1.9199	1.8965	0.82%	0.39%	1.24%	0.01%	0.0207	1.09%
Day 3, analyst 2	1	0.705	0.7042	0.7061	0.7048	0.705	0.11%	0.16%	0.04%	0.00%	0.001	0.14%
	2	1.6174	1.6388	1.6335	1.6373	1.6365	1.32%	0.99%	1.23%	1.18%	0.0027	0.16%
	3	1.7728	1.7905	1.7885	1.7724	1.7838	1.00%	0.89%	0.02%	0.62%	0.0099	0.56%
	4	1.8508	1.8771	1.8769	1.8684	1.8741	1.42%	1.41%	0.95%	1.26%	0.005	0.26%
	5	1.8806	1.9151	1.9117	1.9209	1.9159	1.83%	1.65%	2.15%	1.88%	0.0047	0.24%
	6	1.9095	1.9532	1.9713	1.9708	1.9651	2.28%	3.23%	3.21%	2.91%	0.0103	0.53%
	7	1.9136	1.9918	1.9794	1.9613	1.9775	4.09%	3.44%	2.49%	3.34%	0.0154	0.78%
	8	1.9173	1.9064	1.9211	1.9246	1.9173	0.57%	0.20%	0.38%	0.00%	0.0097	0.51%
Day 4, analyst 2	1	0.7116	0.712	0.7115	0.7112	0.7115	0.06%	0.01%	0.05%	0.00%	0.0004	0.05%
	2	1.6293	1.6492	1.6425	1.6457	1.6458	1.22%	0.82%	1.01%	1.02%	0.0033	0.20%
	3	1.7891	1.8087	1.8012	1.7997	1.8032	1.10%	0.68%	0.60%	0.79%	0.0048	0.27%
	4	1.8697	1.8956	1.9	1.9026	1.8994	1.38%	1.62%	1.76%	1.59%	0.0036	0.19%
	5	1.9005	1.9267	1.933	1.9283	1.9293	1.37%	1.71%	1.46%	1.51%	0.0033	0.17%
	6	1.9306	1.9912	1.998	1.9911	1.9934	3.14%	3.49%	3.13%	3.26%	0.004	0.20%
	7	1.9348	1.9648	1.9842	1.9806	1.9765	1.55%	2.56%	2.37%	2.16%	0.0104	0.52%
	8	1.9386	1.9275	1.9546	1.9339	1.9387	0.58%	0.83%	0.24%	0.00%	0.0142	0.73%

TABLE 4

Average R^2 at 260 nm and 280 nm of each purity level. The measurements demonstrate great linear correlation with Beer's law.

Dilution level	Day 1, analyst 1		Day 2, analyst 1		Day 3, analyst 2		Day 4, analyst 2	
	R^2_{260}	R^2_{280}	R^2_{260}	R^2_{280}	R^2_{260}	R^2_{280}	R^2_{260}	R^2_{280}
1	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
2	1.0000	0.9999	1.0000	0.9999	1.0000	0.9999	1.0000	1.0000
3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
4	1.0000	0.9999	1.0000	1.0000	1.0000	0.9999	1.0000	0.9999
5	1.0000	0.9999	1.0000	0.9999	1.0000	0.9999	1.0000	0.9999
6	1.0000	0.9999	1.0000	0.9999	1.0000	0.9999	1.0000	0.9999
7	0.9999	0.9999	1.0000	0.9999	0.9999	0.9999	1.0000	0.9999
8	1.0000	0.9999	1.0000	0.9999	1.0000	0.9999	0.9999	0.9999

showed the best linear correlation with an R2 of 0.999 (Figure 2). The results of the linear regression analysis show that the method is found to be linear.

Accuracy

Accuracy of the DNA purity method was inferred from the linearity, repeatability, and intermediate precision. The acceptance criteria for each of the studies was met. As a result, the Slope Spectroscopy method is considered accurate for determining DNA purity ratios.

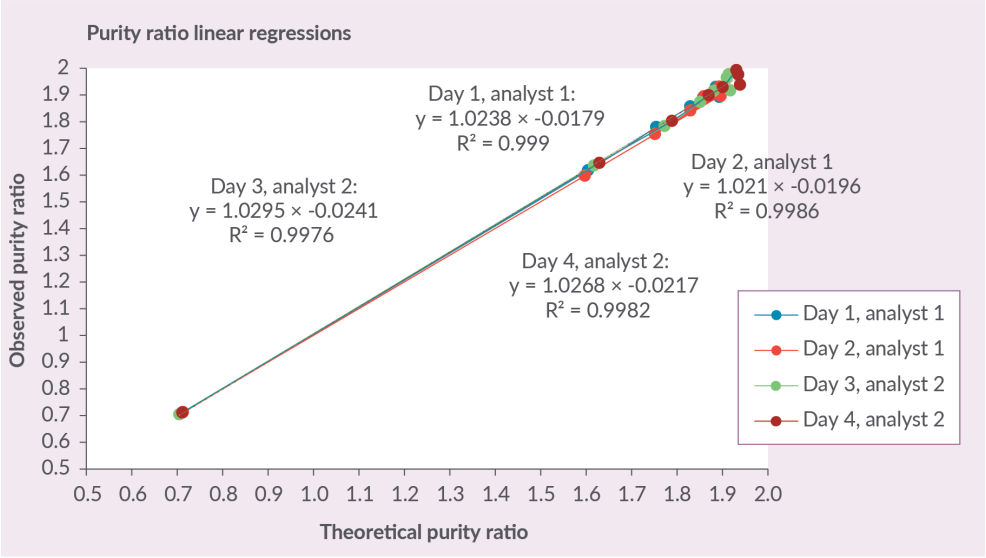
DISCUSSION

The specificity was the first study to be performed to determine if baseline correction was required for subsequent analyses. The results of the buffer demonstrated that the DNase/RNase-free distilled water had little to no absorbance, meaning there is no interference from the buffer on the absorbance of the DNA.

The intermediate precision study demonstrated strong correlation between the theoretical purity ratios and the average observed purity ratios. Even with testing over the course of 4 different days and two different

FIGURE 2

Linear regressions of the theoretical purity ratios against the observed purity ratios. The figure demonstrates great linear correlation between the datasets.



DOI: 10.18609/cgti.2022.155

analysts, the SoloVPE System was able to demonstrate reproducible results. The highest percent error that was measured on each day was 2.77%, 2.06%, 3.34%, and 3.26%, demonstrating that the SoloVPE System was able to accurately determine the theoretical purity ratios. Additionally, the majority of those measurements that exhibited a higher percent error could be related to dilution error.

Furthermore, the repeatability study demonstrated that the SoloVPE method is repeatable. All triplicate readings at each purity level resulted in %RSD values much lower than 2.00%. With the exception of one purity level, all triplicate readings were under 1%, demonstrating the SoloVPE System's ability to produce repeatable results. Moreover, the linearity study demonstrated that the measurements were correlating well with Beer's law. Each measurement at 260 nm and 280 nm demonstrated an R2 ≥ 0.999. The theoretical purity ratios also showed great linear correlation against the average observed purity ratios. Almost all measurements achieved an R2 of 0.999, with the lowest R2 being 0.9976 (Figure 2). All studies indicate that the SoloVPE method is accurate to support DNA purity ratio determination.

Throughout the study, it was interesting to see that the purity level decreases after the 90% DNA solution. Theoretically, the 100% DNA should have the highest purity ratio; however, this phenomenon happens consistently for each measurement and is independent of the rep, analyst, and day. It is difficult to pinpoint the exact reasoning for this; however, the slope of the 100% DNA at 280 nm was consistently the highest. Therefore, either the > 90% DNA is so concentrated that the protein absorbance is increasing, thus lowering the ratio, or there is a lack of protein absorbance contributing to the DNA at these high levels, which would also lower the ratio. Overall, all purity ratios were well above their acceptance criteria. Additional testing is required to come to an accurate conclusion.

The SoloVPE is a convenient tool to carry-out DNA purity studies. It does not require sample dilution which helps save time. Traditional UV-Vis spectroscopy requires background correction, however for the SoloVPE System, if the media demonstrates a slope lower than 0.01 Abs/mm, background correction is not essential. For concentration calculations (DNA and proteins), merely entering the extinction coefficient and wavelength will produce the results. The user result section makes it easy to carry out other calculations using the SoloVPE System, which are generated along with the data, thus barring the need to use excel sheets and validating them.

CONCLUSION

This publication demonstrates the SoloVPE System as the preferred method of DNA purity determination. Regarding this application, the Slope Spectroscopy method allows the SoloVPE System to overcome the issues that are seen with traditional spectrophotometers. The SoloVPE System passed all acceptance criteria, concluding that the method is specific, precise, repeatable, linear, and accurate. The SoloVPE System has proven to be a reliable analytical method to support DNA purity determination.

REFERENCES

1. Huss VAR, Festl, H, Schleifer KH. Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst. Appl. Microbiol.* 1983; 4(2), 184–192.
2. Glasel JA. Validity of nucleic acid purities monitored by 260nm/280nm absorbance ratios. *Biotechniques* 1995; 18(1), 62–63.
3. Sambrook JF, Russell. DW. *Molecular Cloning: A Laboratory Manual (3rd ed.).* 2001, Cold Spring Harbor Laboratory Press.
4. Nucleic Acid Quantitation - an overview | ScienceDirect Topics. ScienceDirect. (Accessed Jun 2022)

5. Matlock B. Assessment of Nucleic Acid Purity. Thermo Scientific 2015. (Accessed Jun 2022)

6. Jones-Goldstein DM, Jones M, Haydu S. Determination of plasmid DNA purity ratio in human gene therapy products using slope spectroscopy method. Repligen Corporation 2021 (Feb 22). (Accessed Jun 2022)

AFFILIATIONS

Nigel Herbert
Senior Bioanalytics Applications Specialist,
Repligen Corporation

Drusha Purohit
Scientist I, Analytical – Compendial Group,
Charles River Laboratories

Hannah Mignault
Team Lead, Analytical – Compendial Group, Charles River Laboratories.

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: *The authors would like to acknowledge Joe Ferraiolo, At-Line Applications Director, Bioprocess Analytics, Repligen Corporation and Scott Stone, Senior Scientist, Analytical Services, Charles River Laboratories*

Disclosure and potential conflicts of interest: *Nigel Herbert discloses that virtual meetings were conducted with Charles River Labs. Drusha Purohit discloses data was provided and reviewed by Charles River Labs and it was written by Repligen. Drusha Purohit and Hannah Mignault disclose virtual meetings were conducted between Charles River Labs and Repligen. 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: *Jul 28 2022*; ***Revised manuscript received:*** *Aug 24 2022*; ***Publication date:*** *Jul 14 2022.*

REPLIGEN

DOI: 10.18609/cgti.2022.155

INTERVIEW

Insights into the editing of the human genome: where can novel non-viral polymeric delivery agents take us

Charlotte Barker, Editor, *BioInsights*, talks to **Tom Foti**, Group Leader for Plasma Engineering & Production &, **Kris Saha**, Associate Professor of Biomedical Engineering, Medical History and Bioethics, University of Wisconsin

TOM FOTI has more than 25 years of biotechnology experience and serves as Vice President/GM of the Protein Business Unit. He was one of the original founders of Aldevron's protein services business, which was formed in 2009. Prior to working for Aldevron, Foti served in several roles in the Merck KGaA Bioscience Division, most recently as the Director of its Global Custom Services Business. He started his career in 1992, with Novagen, Inc. serving in manufacturing and operational roles until 1999. He played college basketball while earning a Bachelor's of Science in Biotechnology and Microbiology from North Dakota State University. Foti also holds a Master's in Business Administration from Edgewood College and a Management Leadership Certificate from Massachusetts Institute of Technology.

KRIS SAHA is an Associate Professor of Biomedical Engineering and Medical History and Bioethics at the University of Wisconsin-Madison. He was recently named the McPherson Eye Research Institute's Retina Research Foundation Kathryn and Latimer Murfee Chair for 2019-2022. His lab is at the Wisconsin Institute for Discovery (WID), and he participates on campus in the executive committees of the Stem Cell and Regenerative Medicine Center, Robert F. Holtz Center on Science and Technology Studies, and Forward Bio Institute. Prior to his arrival in Madison, Dr. Saha studied chemical engineering and biotechnology at Cornell University, University of Cambridge, and the University of California, Berkeley. In 2007 he became a Society in Science: Branco-Weiss fellow in the laboratory of Professor Rudolf Jaenisch at the Whitehead Institute for Biomedical Research at MIT and in the Science and Technology Studies program at Harvard University with Professor Sheila Jasanoff in Cambridge, Massachusetts. At UW-Madison, major thrusts of his lab involve gene editing and cell engineering of human cells found in the retina, central nervous system, liver, and blood. He has published more than 75 scientific manuscripts, filed several patents, and received awards that include the National Science Foundation CAREER Award, Biomedical Engineering Society's Rising Star Award, and Gund Harrington Scholar Award. He is the leader of the gene therapy biomanufacturing impact area of the Grainger Institute for Engineering, a member of the National Academies' Forum on Regenerative Medicine, a co-lead for the T cell testbed within the National Science Foundation's Center for Cell Manufacturing Technologies (CMaT) and on the Executive Committee of the National Institutes for Health's Somatic Cell Genome Editing (SCGE) Consortium.

Cell & Gene Therapy Insights 2022; 8(8), 1005–1013

DOI: 10.18609/cgti.2022.149

Q Kris, tell us about the work you are doing in your lab and in particular, your associations with the NIH Somatic Cell Genome Editing Consortium (SCGE), and with the University of Wisconsin Health Program for Advanced Cell Therapy (PACT)

KS: My lab is focused on developing new cell and gene therapies, namely using new genome editing tools. We are situated at the University of Wisconsin (UW), Madison, in a multidisciplinary center called the Wisconsin Institute for Discovery. We take advantage of fantastic resources on-campus, from clinical partners, science partners, engineering partners, manufacturing partners, and the rich ecosystem of the Madison biotech community.

One of our principal ideas is manufacturing genome editors in a more streamlined fashion, as a plug and play platform technology. We can program it to various parts of the genome safely and with high potency, within either a cell that we are manipulating outside the body, or within a tissue inside a patient.

Currently, many of the challenges have been how to deliver these types of genome editing machinery, namely clustered regularly interspaced short palindromic repeats (CRISPR), into the right cell at the right time with the right effect. Thus far, these have been very large

molecules, in terms of drugs that we would like to administer. There is a large challenge in terms of delivery of these components into the cell. Engineers and biologists have traditionally used engineered viral vectors to deliver these types of large proteins, RNA, and DNA, which has plenty of advantages.

Our approach in my lab has been to look for alternatives that could avoid the use of viral vectors. Much of our work thus far has been to use engineered proteins, RNA, and DNA, without any viruses, such that we can deliver them into blood cells, T cells, natural killer (NK) cells, and stem cells to enable genome edited cell products that can be infused into a patient.

The other part of the lab has been thinking about packaging proteins, RNA, and DNA into non-viral systems such as nanoparticles made from polymers and other synthetic components, such that they can travel in the bloodstream to a particular tissue and deliver the genome editing payload. For example, upon injection into either the brain or the back of the eye, they hit the right types of neurons or other supporting structures that would produce a therapeutic effect.

This work is an approach that requires team science. We leverage work in the field by colleagues and collaborators. We talk to clinicians and industry, including biologists and geneticists often. One of the exciting parts of working in this field is being able to be multilingual in many different disciplines and being able to learn from others thinking about these challenges from a different discipline and perspective.

Both efforts have been leveraging work with two federally funded national centers. One is the Center for Cell Manufacturing Technologies (CCMT), supported by the National Science Foundation (NSF), and headed primarily at Georgia Tech, and here at Wisconsin. We have industry partners and the primary goal is to bring together the right type of team, involving clinicians, engineers, and biologists to manufacture higher quality and safer genome edited cell therapy products.

The second national center is the Somatic Cell Genome Editing Consortium (SCGE) funded through the National Institute for Health (NIH). This is focused primarily on delivering genome editors into the body *in vivo* such that cells are directly edited without removal. The consortium has a rich set of collaborators across the country and has almost completed Phase 1 of its support. It will be launching into phase 2 of the support next year.

Q What has Aldevron done to support Kris' work? How have both parties benefited from that?

TF: We have enjoyed a rich relationship from an innovation standpoint with Kris for at least five years at UW, through Aldevron's site in Madison. The way Aldevron thinks about genomic medicine is that we want to manufacture DNA, RNA, and proteins, both at research stage to collaborate with scientists like Kris, but also as we translate those basic research protocols to a potential clinical setting. Kris and others in translational medicine roles at universities can then continue to progress their research in a coordinated way with high-quality reagents all the way to the clinic.

We collaborate with Kris with research tools we provide, both on the DNA side, working on a Nanoplasmid™ vector project, and on the protein side we have been delivering CRISPR

nucleases. We benefit from this type of collaboration as innovation defines our future as one of the values of Danaher. We need to innovate not just internally, but also with universities such as UW. We are looking forward to continuing to see Kris' work translate into the clinic and hopefully to curative therapies.

Q The SCGE has goals around gene editors, delivery technologies, methods for tracking edited cells ***in vivo***, and developing new animal and human models. Which of these do you see as the biggest challenge and why?

KS: Delivery is a big challenge and has been a primary focus of Phase 1 of the consortium. There was a saying in the field that the three major problems are delivery, delivery, delivery. There has been fantastic progress, for instance hidden delivery with genome editors is in many ways a solved problem. However, there are many other diseases and disorders out there that affect the brain, the eye, and complex tissues like the heart. There is room to be able to deliver, not only the traditional nucleases but some exciting new editors that have been developed, such as base editors, prime editors, and mitochondrial editors. The delivery challenges for each of those is unique.

One of the interesting ideas that we are playing with within the consortium is how to make these different components of a drug – the delivery system and the editor – work as a platform. Once we spend significant effort to deliver something to the back of the eye for instance with high efficiency and good safety, how hard is it to take that same delivery system and change the editor to hit a different target?

Phase 1 has been primarily focused on making new tools. We will release a toolkit that releases the data publicly to researchers who want to hit a particular cell type in the body for a specific indication. In theory, they would be able to identify a delivery system and editor that could be combined relatively straightforwardly, and the toolkit will allow them to edit that cell type in a therapeutic fashion. We have some exciting projects in the kidney, brain, heart, and lungs that will be eventually released in the toolkit.

The next phase is having an impact in the clinic. There, the work with companies like Aldevron and Danaher is essential, because to do this type of work in an academic setting is challenging. It is resource-intensive, and requires reagents, expertise, and collaboration with industry. The SCGE consortium in Phase 2 is going to invest many resources to attempt a few home runs in the clinic, including proof of principle strategies that the field can point to as trailblazing in getting to an Investigational New Drug Application (IND). That is going to drive innovation on the delivery side, as well as innovation in manufacturing and regulatory science to allows us to have that type of impact in the clinic.

Q Tom, where would you see the role of industry in tackling some of those challenges Kris has highlighted?

DOI: 10.18609/cgti.2022.149

TF: One of the reasons that we have had such a good relationship with Kris and his lab, and others in academic settings, is that we want to enable these researchers to realize their dreams for curative therapies.

> "As an industry leader in genome editing...trying to solve the challlenges in delivery"
> - Tom Fotil

Kris was talking about platform manufacture solutions as they relate to developing a curative therapy. There are roughly 8,000 monogenic diseases that could potentially be treated by these curative therapies. The idea with genome editing is to try and create something versatile. Part of the reason we have a great relationship is we have a good alignment over the vision of what we are trying to do. We cannot do it ourselves but coming together we can do amazing things.

We view the scientific problem in different stages. We have the actual high-quality reagents. As an industry leader in genome editing, we are trying to solve the challenges in delivery. Precision NanoSystems, a Danaher company, are collaborating technically with Kris' team to be able to deliver either ribonucleoprotein particles (RNPs) or mRNA to specific tissue types, with specific cargo. We are making reagents that increase robustness on a consistent basis from a manufacturing and a quality standpoint, to enable Kris to translate this into the clinical setting.

One of the ways we as an industry partner help with that is on the quality and regulatory standpoint. We are trying to enable researchers by having drug master files for our reagents. When Kris and his team are filing Investigational New Drug Application (INDs) with the Food and Drug Administration (FDA) for a specific clinical trial, he can refer to Aldevron drug master files at the FDA to make his filings more streamlined.

KS: This is a complementary strategy. We work here with the Program for Advanced Cell Therapy (PACT) which is space in our hospital that can perform GMP-grade cell manufacturing. As a hospital, we are not here to treat hundreds of patients with a new product. Our role primarily is to show proof of concept, usually in a small-scale study and Phase 1 or 2. We want to then spin that out and work with industry to scale that up assuming its safety and efficacy.

We have learned the lesson from the CAR T field that you must spend significant time in that early-stage Phase 1 and 2. If you are able to find game-changing success, that can spur on a whole field, which has certainly been the case in CAR T. We see a lot of value in being able to learn from some of those first-in-human trials.

The resurgence of gene therapy now has taken that role seriously. At UW, even with the unique resource of PACT, we have a small regulatory team of a few people, whereas Aldevron and Danaher have hundreds of people with expertise that can help us move that early-stage technology manufacturing development into larger scales required for Phase 2 and 3.

Q Kris, what would you say is on the horizon for genomic medicine?

KS: We have a lot of momentum in both national centers, as well as in many cities like Madison, where industry is coming together with academia. Phase 2 of the SCGE is focused on clinical impact. There are several promising projects that I believe will get to an IND, likely in the next five years.

There is going to be a focus on figuring out ways to make platform technologies with genome editors to address rare diseases in a more efficient and streamlined way. There are more and more variants being identified, using recent advances in genomics, such as the report of the hundred-dollar genome a few weeks ago. Certainly, there will be more patients that find themselves with potentially new variants that could be the target of genome editing strategies.

The outstanding question that lies at the horizon of genomic medicine is – how do we assemble our tools and come together to be able to make potentially curative therapies for those types of individuals and patients in a streamlined way? Many would argue that the way that we are doing development now, where particular therapies end up costing hundreds of thousands of dollars, is something that is likely not sustainable for the entire US or even global population. There is going to be a lot of innovation there which I am excited to see.

Similarly, the CCMT is focused on clinical impact with clinical and industry partners. We are starting to put in in-line sensors during manufacturing to get almost instant readouts of quality that can help us tune the manufacturing process to make more of the right cell that potentially could be more potent and curative. Those are exciting prospects in addressing some of the quality and cost considerations for *ex vivo* gene edited products.

Q Aldevron recently became a Danaher company. Tom, what is Danaher's role in advancing genomic medicine? What is on the horizon from your perspective?

> "...to be a focus on figuring out ways to make platform technologies with genome editors to address rare diseases in a more effcient and streamlined way"
> - Kris Saha

TF: We are excited about the future. We have long participated in the cell and gene therapy world: Aldevron has been a leader in producing plasmid DNA, both at research-grade and GMP, and many of the current clinical trials in gene therapy use Aldevron-manufactured plasmids.

As we continue to think about how science has changed, we see that mRNA and proteins are equally important in trying to deliver the entire solution. We have been working on this for quite some time, and we have three

DOI: 10.18609/cgti.2022.149

platforms from research-grade to GMP. In August 2021, Danaher purchased Aldevron, which opened up a much larger horizon of tools for our use.

When we think about genomic medicine now and the vision of where we are going, Danaher has a genome medicine organizational structure in which Aldevron operates. We think about the value across the entire scientific chain from technologies enabling the original sequence of what you want to correct all the way to manufacturing for the patient in a fast and standardized way.

When we think about that continuum, we think about DNA, mRNA, and proteins. We can now think about guide RNA from Integrated DNA Technologies (IDT), that can help deliver in a ribonucleoprotein (RNP). We manufactured mRNA, so if you want to introduce a nuclease using mRNA to a target a specific tissue, we can help support those programs. Lipid nanoparticles (LNPs) are another of the delivery solutions, and the LNPs that are made by a Danaher company, Precision NanoSystems, allow for delivery of both mRNA and RNPs.

We also talked about fill and finish as an important component. After you get through Phase 1 and Phase 2 with clinical trials containing around 20 patients, if you want to convert that basic therapy into an FDA-approved therapy that can reach the masses, you need fill and finish capabilities and strong analytical platforms. Some of these analytical platforms are also Danaher companies, such as Beckman Coulter, Molecular Devices, SCIEX, and Phenomenex. All these analytical platforms can help for the release of GMP manufactured products, so that product quality and patient safety are always at the forefront.

Another thing that excites me about the future is research in rare diseases. Historically, pharma and industry have not focused on rare disease because from an R&D perspective, they were looking at bigger markets and bigger patient populations. The idea of platform curative therapies is possible with genomic medicine, and there are many universities thinking about N-of-1 programs, including Fyodor Urnov of the Innovative Genomics Institute (IGI) at University of California, Berkeley. We are excited about those types of programs also, because it is so difficult for families with children with rare diseases who do not get the support from industry versus bigger patient populations. The idea of curative medicines for rare diseases is enticing.

AFFILIATIONS

Tom Foti
Vice President, Protein Business Unit, Aldevron

Kris Saha
Associate Professor of Biomedical Engineering and Medical History and Bioethics, University of Wisconsin

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 Saha's lab conducts sponsored research from Synthego and Spotlight Therapeutics. He has received royalties from from WARF and the University of California.*

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

ARTICLE & COPYRIGHT INFORMATION

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

Interview conducted: *Jul 29 2022; Publication date: Sep 13 2022*

We hope you enjoyed reading this interview.
You can also listen to the recorded podcast here:

LISTEN NOW

DOI: 10.18609/cgti.2022.149

Custom Manufacturing for Cell and Gene Therapy

Research to commercial supply of high-quality plasmid DNA, RNA, and Protein

- Over 24 years of manufacturing experience
- ~ 3,000 lots produced for cGMP manufacture of cell and gene therapies
- 140,000+ lots produced for research, clinical trials, and commercial products
- Extensive experience in consistently achieving lots released at the 25g+scale (supercoiled and linear pDNA)
- Standardized, in-stock products at research and clinical grades for viral vector manufacturing, gene editing, and more

aldevron®

The basis for breakthroughs

Contact us today to get started.

4055 41st Avenue South, Fargo, ND 58104 USA
+1 (701) 297-9256
Toll-free (U.S. and Canada): +1 (877) 787-3362
aldevron.com

FASTFACTS

A closed, modular approach to autologous CAR T cell therapy manufacturing

Jason Isaacson, Scientist, Thermo Fisher Scientific

The complex, multi-step process of generating functional CAR T cells includes cell isolation, activation, modification, expansion and finally cryopreservation. Introducing closed processes to replace manual manipulations can reduce contamination, errors, and variability. Closed, modular, automatable instrumentation for specific unit operations within the workflow can improve upon consistency, purity and safety of the final CAR T product. Additionally, scalable and compliant platforms support the transition from early discovery to commercial scale manufacturing.
This demonstration provides in-depth understanding of Thermo Fisher Scientific's digitally compatible, GMP-compliant manufacturing platform to produce CAR T cells.

Cell & Gene Therapy Insights 2022; 8(8), 1045; DOI: 10.18609/cgti.2022.154

CELL PROCESSING

Featuring the CTS™ Rotea™ Counterflow Centrifugation System

Counterflow centrifugation suspends cells in a fluidized bed by exerting a constant flow force against centrifugal forces ensuring cells are gently concentrated without ever forming a pellet in the cone and washed efficiently at unmatched recovery rates (>95% cell recovery).

CTS Rotea instrument: compact, versatile, and can be easily integrated into multiple points within your workflow

Single use kit: compatible with Luer connectors and sterile welding to the fluid containers to help to ensure a closed system

CTS Rotea software: intuitive user programmable interface allows you to view and modify protocols and visually monitor the process in real time

CELL ISOLATION

Featuring the CTS™ DynaMag™ Magnet

Ideal for magnetic isolation in closed, sterile blood bags
Scalable volumes: 50 to 330 mL in static separations and >10 L in continuous flow separations following T-cell expansion protocols

Residual beads that escape initial magnetic capture are retained on a secondary magnet

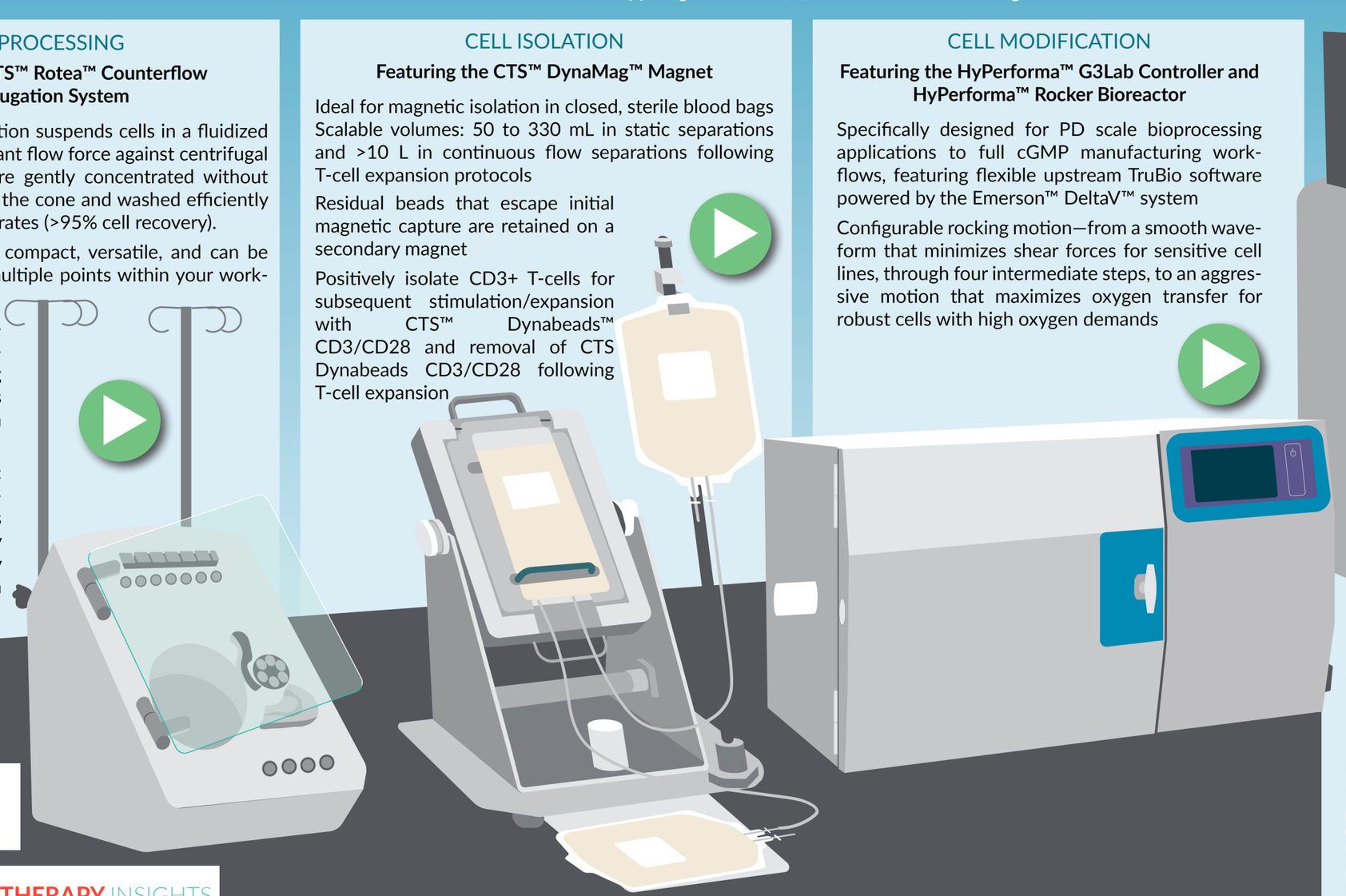
Positively isolate CD3+ T-cells for subsequent stimulation/expansion with CTS™ Dynabeads™ CD3/CD28 and removal of CTS Dynabeads CD3/CD28 following T-cell expansion

CELL MODIFICATION

Featuring the HyPerforma™ G3Lab Controller and HyPerforma™ Rocker Bioreactor

Specifically designed for PD scale bioprocessing applications to full cGMP manufacturing workflows, featuring flexible upstream TruBio software powered by the Emerson™ DeltaV™ system

Configurable rocking motion—from a smooth waveform that minimizes shear forces for sensitive cell lines, through four intermediate steps, to an aggressive motion that maximizes oxygen transfer for robust cells with high oxygen demands



CRYOPRESERVATION

Featuring CryoMed™ Controlled-Rate Freezer

Designed with reliability, protection, and data traceability in mind for your sample preparation and GMP production needs

Intuitive touchscreen display allows for easy set-up, operation, and review of a freezing run. Features six pre-set freezing profiles and space for up to 14 user-defined, "custom" freeze profiles

In partnership with

ThermoFisher SCIENTIFIC

CELL & GENE THERAPY INSIGHTS

FASTFACTS

Robust quantitation of residual host cell and plasmid DNA & oncogenic fragments in HEK-based viral vector manufacturing

Jonas Buege, Thermo Fisher Scientific

Typical adeno-associated viral (AAV) vector manufacturing processes are required to meet current regulatory guidance on residual DNA in order to reduce the oncogenic potential for patients. The resDNASEQ™ system is an all-inclusive system with highly characterized DNA standards and all reagents necessary for residual DNA quantitation, to provide reliable data within 5 h.

RESIDUAL DNA IN AAV WORKFLOWS

Optimization of the manufacturing process is recommended to reduce residual DNA to less than 10ng per therapeutic dose, thus removing oncogenic potential in the final product and ensuring the product meets regulatory guidelines. Testing for residual DNA occurs during process development, during downstream process, and in final QC **(Figure 1)**.

Residual fragment length analysis is expected to demonstrate <200bp, in regulations set by the FDA and the WHO. Oncogenic sequences are of particular concern, especially in viral vector products based on HEK293 and HEK293T cell lines which contain the E1A oncogene.

RESIDUAL DNA KITS FOR HEK293 PRODUCTION

The kanamycin-resistance gene plasmid DNA kit and HEK293 DNA kit can be used throughout the downstream process to support the optimization of a purification process. The E1A fragment length kit can be used to assess the efficiency of a DNA size reduction step, and at the final stages of downstream processing. The assays have been validated using both of our recommended instruments, the Applied Biosystem™7500 Fast and the QuantStudio™5. In addition, all of our resDNASEQ assays have been shown to provide accurate results with various sample matrices.

CUSTOMER DATA FOR AAV PRODUCTION USING HEK CELL LINES

Our residual DNA and fragment sizing assays are suitable for validation per industry expectations to support process development and lot-release quality control (QC).

Figure 2 shows that residual HEK DNA is dependent on therapeutic dosage. It is paramount to have an accurate and sensitive analytical tool that is representative of its analytic target. Our HEK assay is designed to target a highly repetitive element across the entire genome.

Data from one customer's usage of our assay to detect residual DNA fragment length is outlined in **Figure 3.**

Our worldwide technical support network can support you throughout all phases of the implementation process, from early evaluation over qualification and validation all the way to routine testing.

Figure 1. A typical AAV workflow.

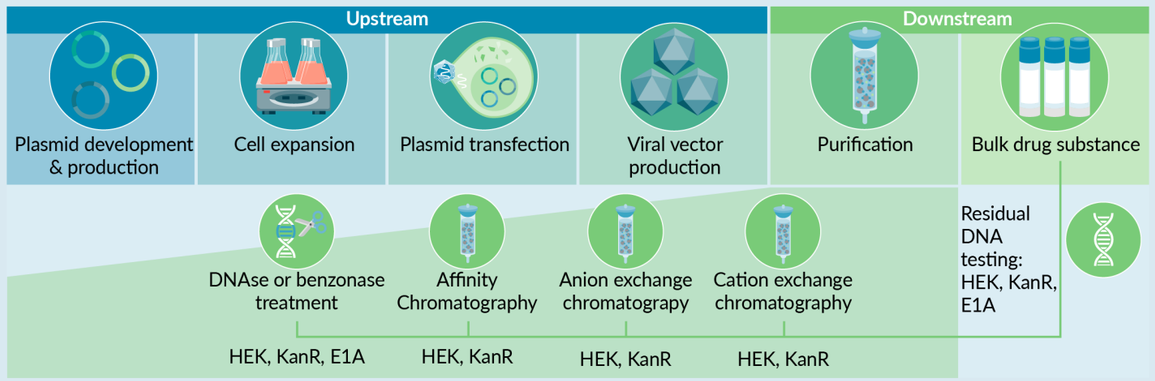


Figure 2. Residual HEK DNA at typical therapeutic dose levels.

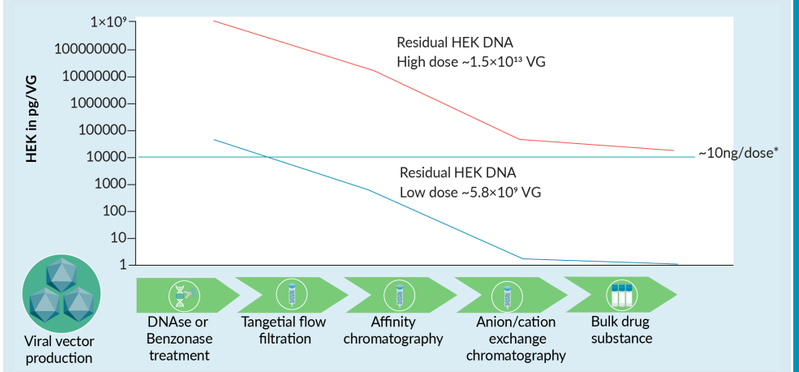
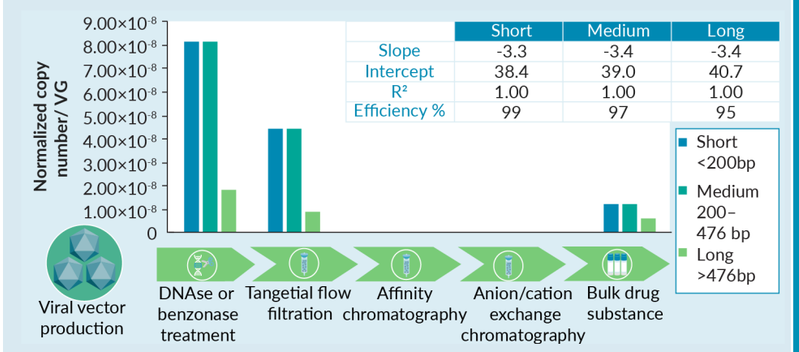


Figure 3. Residual DNA fragment size reduction throughout downstream process.



CELL & GENE THERAPY INSIGHTS

Cell & Gene Therapy Insights 2022; 8(8), 1245; DOI: 10.18609/cgti.2022.182

In collaboration with: ThermoFisher SCIENTIFIC

CELL & GENE THERAPY INSIGHTS

Analytics Channel

CELL & GENE THERAPY INSIGHTS

ANALYTICS CHANNEL

CHANNEL CONTENT

INTERVIEW

Innovation in analytical tool development across the mRNA vaccine & AAV gene therapy spaces

David McCall, Commissioning Editor, *BioInsights*, talks to **Lawrence Thompson**, Associate Research Fellow & Group Leader, Analytical R&D, Pfizer.

LAWRENCE THOMPSON, PhD is an Associate Research Fellow & Group Leader in Analytical R&D within Biotherapeutic Pharmaceutical Sciences at Pfizer. He has been with Pfizer for more than 8 years and is a CMC analytical SME for Pfizer's adenoviral & plasmid DNA based immunotherapeutic, mRNA vaccine drug substance and nucleic acid starting material pipeline (used for both rAAV gene therapy & mRNA vaccine). Prior to joining Pfizer, he spent 3 years in small biotech at two different companies as lead scientist in the development of serum-based cancer diagnostics. He received his PhD in Biochemistry from Vanderbilt University and did his post-doctoral work at the University of Tennessee. His work has generated a number of peer reviewed publications and presentations at scientific conferences as well as internally within Pfizer

Cell & Gene Therapy Insights 2022; 8(8), 1065–1069

DOI: 10.18609/cgti.2022.157

Q What are you working on right now?

LH: Right now, I am working on mRNA vaccines – specifically, the DNA starting material components and mRNA vaccine drug substance. I am in the analytical group supporting the manufacturing of material for clinical trials, before the methods are transferred to our commercial manufacturers.

> "In both the AAV and the mRNA vaccine spaces, I think we are going to see many breakthroughs in multi-attribute detection."

For several years I also worked on adenovirus, which was our early carrier for many cancer vaccines that are also gene therapies. I also helped build our plasmid platform to support our adeno-associated virus (AAV) manufacture for those gene therapy modalities.

Q Innovation in analytical tools is obviously an important requirement for the viral vector-driven gene therapy field. What learnings can be taken from the swift development seen in the mRNA vaccine space?

LH: From a high-level viewpoint, all the modalities that take any gene and transfer it into a host where that gene is then expressed share many commonalities. In AAV, trying to fix a deleterious gene can be like trying to express a protein to cause an immune response in an mRNA vaccine: you have a gene which must be delivered into the body to cause its effect.

The interesting thing about mRNA is that its production is simpler than AAV, because we can control the *in vitro* transcription reaction to make RNA. Producing AAV is a complex process involving dealing with impurities. RNA has some of its own simplicities that accelerate time to clinic, which is the reason many people are interested in it. AAV is more difficult, with longer clinical trials. With mRNA vaccines you have healthy patients in clinical trials, whereas with these gene therapies, you are working with very sick patients.

Despite these differences, many of the analytics and the concepts can be shared, and many of the questions being asked are the same. This is where the next-generation sequencing (NGS) tools come in.

Q In terms of the current state of innovation in bio analytics, what are some of the key, recent breakthroughs for you, and what are they allowing you to do in practice?

LH: In both the AAV and the mRNA vaccine spaces, I think we are going to see many breakthroughs in multi-attribute detection.

DOI: 10.18609/cgti.2022.157

Mass spectrometry has value and impact when looking at the protein component, as it allows measurements of multiple aspects simultaneously. This allows us to get rid of many assays. In addition, NGS can give you a high level of information about the nucleotide payload. You can sequence everything to give a single picture.

There has been much innovation in tools for the bioinformatic analysis of data. The digital strategy ties in with the big data we create with these multi-attribute techniques. Previously, we were not able to probe all of this data, but we are learning more and more in the space. As we bring these bioinformatic technologies forward, we will learn a lot more from the data we produce than what we did in the last couple of years.

Q How can these innovations in bio analytics be made more affordable and QC-friendly?

LH: The prices are coming down. Fourth-generation nano-space tools, such as those from Oxford Nanopore, are always going to be complex, but they are getting better, and are becoming more ubiquitous and widely applied. However, they are still a long way from good manufacturing practice (GMP).

In my opinion, the fastest way to reach GMP is to have your own technology team that takes the NGS and put it inside its own box and build it to be GMP-compliant. Many of the companies that are selling these instruments are not selling them for GMP applications, but that is where the field is moving.

If you want to have real impact, release testing is the way to go. If you can use NGS for adventitious virus testing, for instance, which you need for many of these types of products, then you will no longer have to undertake animal testing. It is much better than animal testing for many reasons.

Q What are the most pressing elements that are still missing from the toolkit? What's top of your wish list?

LH: Moving some of these new tools into the GMP space is definitely something that we need to get done. It's a gap that everyone is working on.

When it comes to AAV, one of the biggest areas of investigation is intermediate particles. Full particles contain the intact genome, whilst empty particles do not. However, the components of the intermediate species need further definition. People are asking many questions about this, and a great deal of work is being done on these intermediate particles.

There remains a lot to be understood in the cell therapy space. When harvesting cells from people that are heterogenous to begin with, and then manipulating them, you see a highly complex machine beyond anything we have done before. It can be a challenge to fully understand the testing, characterization, and comparability. The meaning of comparability for a cell therapy is still under question.

Q Potency has been a recent big topic in gene therapy. What are the chief learnings that might be taken from recent regulatory setbacks in the space?

LH: In general, it is better to plan for more rather than less. You may go in with a streamlined plan for potency where you may get expression and some activity, but it is key to plan for more.

Also, start potency assay development as early as is reasonably possible. Make early engagements with agencies and authorities surrounding your plan.

Q Your work spans several fields, including vaccines and gene therapy. What are some important learnings or repurposed analytical tools that the gene therapy field can take from the more mature sector of vaccines?

LH: As we have discussed, there are many of the same technologies and ideas being shared across the fields of vaccines and gene therapy. It actually goes both ways.

In terms of mRNA vaccine tools being applied to AAV, there is a fragment analyzer tool that looks at the intact RNA and its small particles. That is also being applied to AAV genome integrity. There is also some repurposing of mRNA vaccine tools that can look at both mRNA vaccine and AAV particle size. They do differ in size, but not hugely.

In terms of learnings, many of the vaccines, such as polysaccharide conjugate vaccines, do not correlate exactly with gene therapies. But in AAV gene therapy and mRNA vaccines, with a particle with a nucleotide payload, there is crosstalk with the methods and questions being answered.

Q Finally, what are some key goals and priorities that you have for your work over the coming 12–24 months?

LH: A major goal as we move these multivalent mRNA vaccines to the market is taking NGS as a tool into the GMP space, as a multi-attribute method for mRNA vaccine release testing.

As we begin to move towards strain changes and multivalency, questions remain regarding the optimal methods to do so. That includes self-amplifying RNA vaccines, which are also on the horizon, where you

"A major goal as we move these multivalent mRNA vaccines to the market is taking NGS as a tool into the GMP space, as a multi-attribute method for mRNA vaccine release testing"

DOI: 10.18609/cgti.2022.157

can give low doses and the RNA is amplified, thus removing any concerns around dosage. Also on the horizon are combination vaccines.

AFFILIATION

Lawrence Thompson, PhD
Associate Research Fellow & Group Leader,
Analytical R&D,
Pfizer

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 source: *This article is based on an interview with Larry Thompson carried out on Aug 19 2022.*

Interview held: *Aug 19 2022;* ***Revised manuscript received:*** *Sep 13 2022;* ***Publication date:*** *Sep 19 2022.*