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CELL & GENE THERAPY INSIGHTS

SPOTLIGHT ON
Scale-up/-out of cell & gene therapy manufacturing

Guest Editor
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CELL & GENE THERAPY INSIGHTS

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How to achieve both cost and quality goals in plasmid manufacturing

Silvia Orsini, Plasmids Global Subject Matter Expert, Thermo Fisher Scientific

Addressing challenges with plasmid scalability, quality, and production timelines are key to preparing a therapeutic product for commercialization. This poster will showcase key features and quality attributes for GMP Now™ plasmid DNA, and explain how this new option can help in achieving both cost and quality goals in plasmid manufacturing.

DETERMINING PLASMID QUALITY REQUIREMENTS

The growth of cell and gene therapy and the rapid emergence of the mRNA vaccine market have created intense pressure on plasmid DNA (pDNA) manufacturing. A recent industry report indicates the pDNA manufacturing market may see >20% growth by 2030 [1].

Factors involved in determining pDNA quality requirements include the type of plasmid application (e.g., as a raw material or a drug product/substance), and regulatory guidance (considering a specific program's drug designation). Other factors include the project timeline, funding, risk threshold, and internal regulatory feedback.

ADDRESSING AN UNCERTAIN REGULATORY ENVIRONMENT

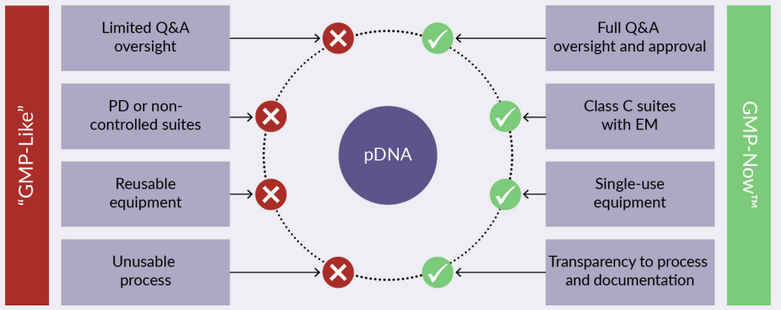
Very little regulatory guidance exists specifically for the manufacture of cGMP raw materials used in cell and gene therapy. Existing guidelines have multiple interpretations and there is a lack of standardization for critical quality attributes and definitions for raw materials. Therefore, pDNA manufacturers must determine the level of controls put in place whilst maintaining a robust supply chain.

Figure 1. EMA recommended standards.

Example products	Application of GMP to manufacturing steps is shown in blue; GMP principles should be applied where shown in yellow Starting material ⟶ Active substance ⟶ Finished product				
***In vivo* gene therapy: mRNA**	Plasmid manufacturing, and linearization	*In vitro* transcription		**mRNA** manufacturing and purification	Formulation, filling
***In vivo* gene therapy: non-viral vector (e.g., naked DNA)**	Plasmid manufacturing	Establishment of bacterial bank (MCB, WCB)		**DNA** manufacturing, fermentation, and purification	Formulation, filling
***In vivo* gene therapy: viral vectors**	Plasmid manufacturing	Establishment of a cell bank (MCB, WCB) and virus seeds when applicable		**Vector** manufacturing and purification	Formulation, filling
***Ex vivo* genetically modified cells**	Donation, procurement; testing of tissues/cells	Establishment of a cell bank (MCB, WCB) for plasmid and/or vector expansion and viral seeds when applicable	Plasmid manufacturing; vector manufacturing	**Genetically modified cells** manufacturing	Formulation, filling

In the table above, the AMTP starting materials are underlined and the AMTP active substance appear in **bold**. The contruction of the plasmid by an *in silico* and molecular biological methods occurs before the plasmid manufacturing and is considered research and development. Therefore it is not under the scope of the current Q&A.

Figure 2. "GMP-like" vs Thermo Fisher Scientific's GMP-Now™ plasmid DNA.



The European Medicines Agency (EMA) recommendations from February 2021 Q&A guidance specifically address plasmids as starting materials **(Figure 1)**. It is recommended that a risk-based approach is used to determine which GMP principles are applicable to the relevant starting material. The use of GMP quality plasmid material can help mitigate the risk of inconsistent batches, which can increase project cost/timelines and present regulatory challenges.

PHASE-APPROPRIATE PLASMID DNA SOLUTIONS

Thermo Fisher Scientific offers flexible pDNA solutions for use in a wide variety of R&D, clinical, and commercial bioprocessing applications, with scale options ranging from 3 L to 1000 L.

The industry has responded to the lack of regulatory guidance/stringency with a number of different "GMP-like" pDNA offerings **(Figure 2)**. Alternatively, Thermo Fisher is pleased to introduce GMP-Now™ pDNA, produced with full application of cGMP practices and with standard documentation provided. This offers a reduced risk of contamination compared to "GMP-like" pDNA and allows for ease of CMC filing, enabling cost and quality goals in plasmid manufacturing to be achieved.

Additionally, Thermo Fisher Scientific provides cGMP pDNA. This material is also produced with full application of cGMP practices but offers enhanced traceability and/or customized documentation for an additional fee.

More details regarding Thermo Fisher Scientific's phase-appropriate service options are represented in **Table 1**.

Table 1. TFS phase-appropriate options for plasmid DNA manufacturing.

Thermo Fisher Scientific phase-appropriate options	GMP-Now™ plasmid DNA (early phase)	cGMP plasmid DNA (early phase–commercial)
Pass-through cost included	●	Estimate provided
Calibrated and qualified equipment	●	●
Produced using Thermo Fisher plasmid platform process	●	●
Produced under full quality oversight	●	●
Produced using quality approved master batch records	●	●
Batches tested using qualified platform methods	●	●
Production in monitored GMP Class C controlled suites	●	●
Produced from MCB	●	●
Client specifications for custom plasmids	●	●
CoA, CoC, TSE/BSE statement provided at release	●	●
Cross contamination control with single-use equipment	●	●
Client audits supported		●
Access to QC raw data		●
Tech transfer custom processes available		●
Process optimization and validation available		●
Executed batch records provided		●
Regulatory support for 3.2.S.2.3		●
Client-specific method qualification/validation		●
Client approval on documentation		●

REFERENCE

1. Plasmid DNA Manufacturing To See Impressive Growth In Years Ahead.

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CELL & GENE THERAPY INSIGHTS

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

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INTERVIEW

Happy medium: considerations in scaling cell culture media strategies

While attractive from an initial cost perspective, performing early development in a cell culture substrate that does not match a therapy's final manufacturing platform can have critical implications on development timelines. In this podcast, **Charlotte Barker,** Editor, BioInsights, speaks to **Dalip Sethi,** Director of Scientific Affairs, Terumo BCT, about best practices for scaling up cell culture substrate.

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Q Cell culture media is a big consideration for therapy developers—does Terumo Blood and Cell Technologies (BCT) have a media offering?

DS: Media is critical to developers because is the vehicle by which cells obtain nutrients, gases, and growth factors, and secrete waste products. Terumo BCT does not have a media offering but our equipment and devices are designed to be cell-type and media-agnostic.

When we are considering protocol development experiments in-house, we consider different media formulations available on the market, as often as possible. We share those results in various methods or channels through our peer-reviewed publications, webinars, posters, and seminars.

Q What are some of the main considerations in choosing a cell culture medium?

DS: One of the main considerations is whether the media can be manufactured at a GMP grade to fully move a process into the clinical scale. Due to cost considerations, a researcher may choose to use the research-grade media to start with, but as they move into clinical and later stages of development, they will need to move to GMP grade. The ideal solution would be either a commercially available formulation that comes in research grade for early development work and GMP grade for manufacturing, or a so-called 'home brew' media formulation that has been tech-transferred to a CDMO for both grades of production.

Another consideration is the type of media that you are using. Does your media contain serum or is it serum free? Is it xeno free? You should consider these factors before moving on to manufacturing. Try to have the best media composition for all cell types that you are growing and avoid animal-derived components. If you cannot go completely xeno-free, there are some further considerations to be made. How will one use a serum substitute without impacting the quality of the cells, including the phenotype of the cells, as one moves from the research to the manufacturing stage? A lot of media companies now provide chemically defined media (CDM) so it is worth considering if there is a CDM that can be utilized to give the best quality cells.

There are many more factors to consider too. For example, in a T-cell culture media, there are multiple components, including proteins, glucose, vitamins, amino acids, trace elements, and inorganic salts. When you are culturing T cells, you should ensure that they maintain the right physiological pH. When cells are growing, they will consume glucose and produce lactate, which can change the pH of the media. To maintain that pH, you need a buffering system. A simple bicarbonate buffering system can maintain pH for a certain concentration of hydrogen ions and lactate, but when you are working with very fast-growing cells, you may need to consider media containing different buffers, such as HEPES.

In addition to the above factors, when we consider T-cell cultures, we cannot forget about interleukins. What kind of interleukins will you be using in your complete media, what will be their sources, and what will be the grade of those interleukins? Again, a researcher may choose to use a research-grade interleukin in the early stages. It will be important to consider if the supplier provides the same cytokines in GMP grade. If the GMP grade is not available, what are the qualifications required as one continues with the research grade?

As you go from a small-scale research development grade to a manufacturing grade, the scale of production will also change. You should have the right supply chain in place so that you can get the media that you need.

There are a lot of questions to think about, and I am sure it keeps researchers up at night!

Q What about media-related considerations when you are using a closed automated bioreactor system—including the in-process analytical components?

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DS: With closed automated bioreactor systems—and I am thinking here about perfusion-based systems—the first thing you must consider is that media have different protein concentrations. In a bioreactor system, you want to make sure that the bioreactor has enough flexibility to accommodate media with different protein concentrations. If you have a low protein concentration medium or a high protein concentration medium (which is sometimes needed to get the right phenotype and culture conditions for a particular cell type), the automated bioreactor systems should be able to handle that without getting clogged or blocked. Having a large membrane surface area helps with that, and it allows you to culture cells with a high-protein medium.

> "Try to have the best media composition for all cell types that you are growing and avoid animal-derived components."

When it comes to process analytical technologies, there are a number of different classes. Off-line analysis involves taking a sample out of your bioreactor system and passing it to a separate QC lab, close by, to test for metabolites, cell phenotype, cell viability, etc. At-line analysis is when the analytical technology is right next to the bioreactor system. In-line analysis is when the process analytical technology is connected in line to the bioreactor system.

It is important to consider how the samples are taken from the bioreactor system and if they are coming with or without a filter. Particularly in the case of high-protein media, you want to make sure that the filter can handle those sampling considerations.

Q Where do equipment and consumables factor into this conversation?

DS: One of the most important factors that any manufacturing equipment provider has to consider is extractables and leachables (E&L). Typically, any surface that encounters the cells—and by extension their media—must go through extensive E&L testing. This ensures that no substances can be drawn out from the substrate into the media to the detriment of cell expansion.

We must also consider interactions between complete cell culture medium and substrate surfaces that, while not toxic, may drastically affect cell expansion. For example, certain materials may have a high affinity for binding to proteins such as cytokines or other growth factors. In this instance, these proteins may bind to and be sequestered from the cells, which may 'starve' them or delay growth curves.

We have also already talked about having the right surface area so that your filter does not get foul or clogged. Filter fouling, wherein the pores of a filter membrane may become clogged with protein over time during media perfusion, is an especially critical consideration for protein-rich formulations such as serum-completed media and even some basal media with high

protein concentrations. Oftentimes this isn't an issue in small-scale testing but can reveal itself upon scaling up.

Q That's very interesting to note... Not the 'first line' issues that you mentioned earlier, but certainly something that can affect therapy development timelines. What does Terumo BCT see as a mitigation strategy?

DS: These are unwelcome surprises to any researcher, to be sure. One of the best ways to avoid them is to use like-for-like substrates from early research to the manufacturing level. The same materials of contact, the same environmental controls, and ideally the same platform should be used. Bioreactors with small volumes and low seeding density requirements that have robust, scaled-up counterparts should be a top consideration for researchers.

Our Quantum Flex is a good example of this: it is one device with two sizes of consumables offered, a small and a standard bioreactor. The small bioreactor is roughly 1/10th of the size of the standard bioreactor and can produce 1B suspension cells or 100M adherent cells under the right culture conditions. This sizing option is great because there is no need to perform a manual pre-culture step in a T-flask or gas-permeable bag—so no substrate changing is required.

The standard bioreactor uses the exact same hollow fibers and materials of contact but can produce 10B suspension cells or 1B adherent cells—again, under the right culture conditions.

There is also data in the literature showing different media types used in Quantum System that resulted in the successful generation of T cells, including Miltenyi's TexMACS™ medium, Irvine Scientific's PRIME-XV T-cell expansion XSFM medium, and Lonza's X-VIVO-15 medium.

Q How would you say your customers' needs are changing as the industry matures?

DS: Customers are increasingly considering xeno-free or serum-free media for potentially smoother regulatory and CMC processes. Manufacturers are also thinking about how to control lot-to-lot variability, CDM is also being discussed in the literature.

Ultimately, the 'happy medium' will come from the combination of the bioreactor and media composition. Getting the right phenotype and number of cells, allowing a cell therapy manufacturer to be able to dose a particular patient or clinical trial participant in the right way, is key.

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BIOGRAPHY

DALIP SETHI PhD currently serves as the scientific lead for Terumo BCT's Cell Therapy Technologies portfolio. He holds a doctorate and conducted post-doctoral studies at Thomas Jefferson University, School of Medicine. In his post-doctoral research, Dalip focused on the development of cancer gene-specific RNA and DNA analogs targeted against cancer genes in the signal transduction pathway for use as cancer diagnostics and therapeutics. Throughout his career in the industry, Dalip has been engaged in developing technologies & methods for use in cell therapy applications. Dalip has authored multiple scientific publications and is a co-inventor on several patents & patent applications. He recently co-authored publications on modular automated systems for $CD3^+$ T-cell manufacturing and monoculture of cord-blood derived $CD34^+$ using an automated, membrane-based dynamic perfusion system. The articles highlighted the benefits of modular automation in cell therapy manufacturing. Dalip is also an ISCT member and participates in committees focused on cold chain, particulates, and process analytical technologies.

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TERUMO BLOOD AND CELL TECHNOLOGIES

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We hope you enjoyed reading this interview.
You can also listen to the recorded podcast here:

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INTERVIEW

Overcoming the challenge of antiviral defense in viral vector manufacturing

Charlotte Barker, Editor, *Cell & Gene Therapy Insights*, speaks to Virica Biotech's **Jean-Simon Diallo**, Scientific Founder/CEO, and **Jondavid De Jong**, Vice President, Scientific Operations. They discuss the current understanding of the mechanisms behind antiviral defenses and consider how the industry needs to react to this problem within the context of viral vector production and manufacturing.

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Q What are you working on right now?

JSD: Right now, we are busy guiding many of our customers on their path to using small molecules to enhance their viral vector or vaccine productivity. We have over 60 projects ongoing, with some leveraging our in-house capabilities for high-throughput virology—the ability to rapidly quantify virus output to test thousands of conditions quickly.

"...it is important to understand that antiviral responses are a product of the specific vector system and the cells being used. The interaction between these two factors is the main determinant of what type of antiviral defense signature, including its kinetics, is involved."
— Jean-Simon Diallo

We are also expanding our lab capabilities as we are seeing an increasing demand for our high-throughput virology platform. Since we have been focused on guiding our clients through all these projects, our internal R&D has been on the back burner. Now, one of our key focuses is increasing capacity to bolster our R&D to support our existing programs and expand on our next-generation technologies.

Q How has the issue of antiviral defenses been assessed in industry-based and large-scale settings?

JDJ: This is an important question because, traditionally, the innate antiviral defenses within the cells used to manufacture viral vectors have been an under-appreciated aspect of the manufacturing paradigm. More recently, Eric Barton's group published a study demonstrating that innate antiviral pathways are very active in HEK suspension cells using traditional triple transfection adeno-associated virus (AAV) production in 50 L bioreactors. They saw induction in two waves: an early-stage induction of interferon and a later-stage induction of interferon-stimulated genes.

It is becoming clear that within these biomanufacturing platforms, the innate antiviral defense pathways are being activated and are likely playing a role in both the amount, as well as the quality of the viral vectors you get out of these biomanufacturing situations.

Q What is the impact of antiviral defenses on viral vector-based cell and gene therapies, and what factors contribute to the innate immune response/immunogenicity?

JSD: The two main impacts are on product yield and product quality. To appreciate that, it is important to understand that antiviral responses are a product of the specific vector system and the cells being used. The interaction between these two factors is the main determinant of what type of antiviral defense signature, including its kinetics, is involved.

There are many factors that can influence this, including those associated with the virus and cells. As many vectors are produced using plasmid transfection, the plasmid quality and

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structure can also have an influence, in addition to how those plasmids and genetic elements enter the cell. There are different receptors that are triggered through different entry mechanisms. The cells evolve clever ways to detect all types of viruses coming at them from many directions.

Furthermore, there is a kinetic element to the innate immune response. There are different waves of triggering antiviral defenses. First, there is the initial onset of either the infection or transfection event, wherein some impurities like lipopolysaccharide (LPS) may remain, especially at a large scale. A first wave of responses is triggered, which will impact the growth of the virus and the quality of the genomes and proteins produced. In the second phase, where the virus is starting to assemble, another round of responses is triggered that will nip at the virus from various directions.

There is a lot that remains to be understood about the way the cell fights against viruses. In the past, everyone assumed that the interferon was doing most of the job, which is why the problem of antiviral defenses was ignored for so long.

Since then, we have learned that there are different types of interferons and other cytokines that have subtle impacts on antiviral responses in different ways. There are hundreds of antiviral effectors out there, not only ones that trigger modifications and digestion of the genetic material but also post-translational modification of the vector itself. This is only now starting to be appreciated, and we are still in the early stages of understanding some of these post-translational modifications. There are many factors of influence.

JDJ: We know a fair bit about the adaptive immune system, but the innate immune system is more of a complex black box right now.

Even small amounts of pathogen-associated molecular patterns (PAMPS), such as LPS, can cause significant problems. We must think of the bioreactor as a community, with cells all signaling each other rather than simply individual cells. Even if the small amount of LPS interacts with one cell, that cell will start sending out signals that act in a paracrine and an autocrine faction. The response happens within the larger community.

Q Current research postulates that antiviral defenses could be rAAV yield and quality modulators. What are these quality metrics, and do they extend beyond what we know? And how does it impact viral productivity?

JDJ: For traditional cell and gene therapy vectors, such as AAV and lentiviruses, many of the quality metrics focus on our full to empty ratio or functional to physical titer. The innate antiviral defenses play a role there.

Antiviral signaling of the effector proteins culminates in the expression of hundreds, if not thousands, of genes and their proteins, whose role is to either shut down the basic processes of the cell to short-circuit viral production or physically alter either the nucleic acid or proteins within the cell. This can have a profound impact, not just on the integrity of the genomes that will be packaged within the vectors but also on post-translational modifications

to the capsid. The impact of these modifications on the potency of vectors remains an open question. In current literature, it has been shown certain modifications that can either help or hinder transduction.

Point mutations and small indels, which certainly could result from the innate antiviral response, will greatly impact the potency of supposedly full vectors. That is something that we are not necessarily detecting with current approaches. The community as a whole is starting to move towards next-generation sequencing (NGS) to get a handle on heterogeneity within those viral populations. This is another interesting aspect where the innate antiviral response could be playing a role.

JSD: Particularly in the AAV space, people often use viral genomes per mL as a standard metric. From my virology perspective, there is a better way of looking at it. The viral genomes are only part of the equation. You need to look at potency and transduction efficiency to ultimately know what is going on. When we build solutions for our clients, this is the primary metric look at.

The other factor is specific productivity. As you improve the upstream yield of your viral vector, you can expect to have lower contaminants in your purified product, particularly for vectors where you need to lyse the cells and release cellular DNA into the media. Improving upstream yield indirectly benefits product quality as long as you have the purification methodologies to deal with that increased upstream productivity. Furthermore, minimizing the amount of plasmid that you are putting in will limit this antiviral defense response, which can have additional benefits in regards to any contaminants being introduced.

Q How is the industry tackling the problem of antiviral defenses?

JSD: We favor a small molecule-based approach that we have been studying for decades, collecting a variety of proprietary molecules that target antiviral defenses in different ways. We like the small molecule approach because it is transient and adaptable to any new situation, typically requiring no real modification to existing processes. This work has led to a library of small molecules we call viral sensitizers or VSE™s, which are used in combination to fundamentally change how cells behave.

There are, however, other ways to deal with antiviral defenses. The most clear alternate route is the genetic engineering of cell lines. The challenge here is that it takes a long time, and the antiviral defense signatures are quite complex and redundant. Some effectors that impact cell viability cannot be knocked out more than transiently at the risk of cell death. Suppose you try genetic engineering approaches to modify those particular pathways. In that case, you may end up with either a tumorigenic cell line or a cell line that takes a long time to proliferate, which will not work in a manufacturing setting. You cannot use the genetic approach to solve all problems, even though CRISPR has been tremendous for the field. There will be certain instances where the small molecule approach will continue to be the only viable approach for many years.

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> "The sheer number of options in terms of the available technologies makes it difficult to standardize. There are many options for cell line lineages, media, transfection reagents, and even the different modalities of nucleic acids."
> **— Jondavid De Jong**

JDJ: Both the genetic and the small molecule approaches are complementary. For example, if someone did develop a genetically engineered cell line with certain innate antiviral defenses knocked out, there would still be redundancies within those pathways. This is where an engineered cell line plus a small molecule strategy could be viable.

Q With many variables at play, what are the challenges associated with standardizing the viral vector manufacturing process, particularly at scale?

JDJ: The sheer number of options in terms of the available technologies makes it difficult to standardize. There are many options for cell line lineages, media, transfection reagents, and even the different modalities of nucleic acids. There is also a wide variety of capsids—some natural, some engineered—and many different transgenes out there.

With all of those variables at play, standardization becomes a challenge. To optimize platforms, a custom approach is required. This involves matching the optimized transfection reagent with the optimized ratio of nucleic acids in a particular cell line. Bringing small molecule additives to push the envelope and maximize the output will require a customized approach rather than a standard one. A standard approach can get you to a certain level, but a customized approach is necessary to ramp up the system.

JSD: The industry is doing a good job of pushing that upper limit continuously and honing in on certain cell lines and transfection reagent combinations that consistently do a good job. Once in a while, somebody will come up with an idea that the cell does not like or that viral biology does not permit. That is where custom solutions will be needed. We need standardized basic solutions to get things going and alternative solutions once products reach commercial scale.

Even a small increase in viral vector yield will provide huge returns on investment when we are talking about applications that may require a 500 L bioreactor run to treat one patient. That is why there is such a need for technologies of all types that continue to boost the standard.

Q What are your key goals and priorities for yourself and Virica Biotech over the next 12–24 months?

JSD: There are many developers out there creating fantastic new therapies for rare diseases and, increasingly, less rare diseases. This is what is fueling our growth. Our technology works across a broad range of platforms, including vaccines and cell and gene therapy. There is a lot for us to do over the next few years.

A key focus for us is continuing to expand the capacity of our high-throughput virology platform. As we identify more small molecule enhancers, our high-throughput capabilities become increasingly important in rapidly providing the best combination of molecules to significantly enhance production. At the same time, that expansion in capacity will be key to allowing Virica to continue to provide new solutions for our growing list of clients, within this increasingly complex manufacturing paradigm.

As we grow and expand, my personal goal is to lead our all-star team. To ensure we continue to meet our clients' goals, there are always opportunities for folks to join the team.

JDJ: We have traditionally focused on viral vector manufacturing and the innate response there. We know that the transduction of cellular-based therapies will also face these same challenges. Primary cells, I would argue, would have a more fully intact innate anti-viral response than HEK cell lines. Over the next 6–12 months, we will explore this avenue further to demonstrate the use of small molecules and these kinds of strategies on the transduction enhancement side.

BIOGRAPHIES

JEAN-SIMON DIALLO is a Scientist with broad expertise in biochemistry, molecular biology, cancer therapeutics and viral immunology. Dr Diallo is an internationally recognized expert in oncolytic virotherapy and was seminal in the discovery of Viral Sensitizers and their development for virus manufacturing and cancer therapy applications in combination with oncolytic viruses.

JONDAVID DE JONG is an industry expert with over 20 years of bench experience in the fields of virology, molecular biology, oncology and nanoparticle research. As Vice President of Scientific Operations at Virica Biotech, he leads a team of highly skilled scientists and business development professionals who are dedicated to helping biotech and CDMO organizations make viral medicines a reality.

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Considerations around the scalability of automated cell therapy manufacturing

The hefty investment required to introduce automation to early-stage processes has traditionally spurred many in the field to postpone that decision. However, is the combination of technological innovation and a more informed sector beginning to turn the tide? **David McCall** (Senior Editor, BioInsights) speaks to **Krishnendu Roy** (Director, NSF Center for Cell Manufacturing Technologies and Marcus Center for Therapeutic Cell Characterization and Manufacturing, Georgia Institute of Technology, and starting August 1, 2023, the Bruce and Brigitt Evans Dean of Engineering at Vanderbilt University) about questions of scalability, and recent progress towards the goal of non-clean room GMP manufacturing with integrated process control for the cell therapy field.

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Q What are you working on right now?

KR: Across both the NSF Center for Cell Manufacturing Technologies and the Marcus Center for Therapeutic Cell Characterization and Manufacturing, we are working on three or four key areas. One is to understand how we can discover critical process parameters (CPPs) and critical quality attributes (CQAs) of cell therapies for specific

disease conditions and cell types, both in terms of understanding processes to be able to predict and control the end product quality, but also understanding the quality matrix of an end product that is most correlative to a patient outcome. That is an important thrust area for us, and it is one where we primarily employ bioinformatics, multiomics analysis, and correlative and predictive modeling tools.

"We want to understand how to use a fully automated system to measure process and product parameters in real time or pseudo-real time while achieving a consistent and desired product quality."

We also have a strong focus on the area of in- and at-process sensors and monitoring, as well as assays. We are looking into various engineering tools, technologies, microfluidic devices, and automation devices, to do assays within the manufacturing context for measuring the process and the product, and also in order to better understand function.

The third area is more at the systems level. We are still developing materials, bioreactors, and supply chain models, but a large part of our focus is on integrated feedback-driven dynamic automation. We are figuring out how we employ sensors and measurement tools in a closed-loop control system and use measurement-driven process control. We want to understand how to use a fully automated system to measure process and product parameters in real time or pseudo-real time while achieving a consistent and desired product quality.

Q What are some key recent developments and the most pressing challenges regarding automation in the context of scale-up and scale-out of cell therapies?

KR: It is an interesting time in the cell and gene therapy space because we have to think about both autologous and allogeneic cell-based therapies. The autologous space is currently focused on large, centralized manufacturing production facilities, as well as the logistics for shipping, handling, collection, and delivery. However, a lot of new companies are starting to gradually shift focus to small footprint distributed manufacturing, or even point of care manufacturing platforms. The focus is on fully closed, automated manufacturing units. There, you are taking a fixed recipe process and you are automating it. There may be a routine measurement here and there, but nothing beyond that. The concept of real-time process control to get to a fixed quality product with process and cell-specific sensors and measurements is still a little way away, but the field is gradually shifting towards that goal, with distributed manufacturing as a potential solution.

The allogeneic space is somewhat different in terms of automation challenges or concepts. In the allogeneic space, people view large-scale centralized manufacturing as being sufficient because you are using a single donor for many patients, which is a model you can scale. The

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main challenges in including automation in that type of process are around reproducibility, reduction of labor, and cost–benefits.

For example, in the induced pluripotent stem cell (iPSC) field, even if one starts with the same master bank cell, variability naturally arises in the process—cellular differentiation is inherently a stochastic process. So, the question becomes: how do we use automation to measure and influence the process to control that variability?

Q How have key stakeholder's viewpoints regarding the practicality and cost–effectiveness of automating advanced therapy manufacturing evolved, particularly at the earlier stages of process development? And what does this mean for the field at large?

KR: In the last 3 or 4 years, automation has become a much more acceptable term in the biotech and pharma industry boardrooms. People are more willing to invest because they see that the cost of labor, the uncertainty in terms of process reproducibility, and the variability in human operators, are all quite significant. Even if it is a fixed recipe automation, which is where the industry currently is, it is still hugely beneficial. Yes, there is an upfront cost, but it is increasingly understood that there are also longer-term benefits in terms of decreased batch failures, better reproducibility, lower COGs (including lower labor costs).

People are slowly incorporating automation earlier and earlier into process development because all of these processes have an impact on the quality of the cells. If you want to do all of your early development studies on a particular process that is highly manual, and then you go into a fully automated process, you may see some differences in cell product quality. You do not want to progress too far in your study and then suddenly switch to an automated process. It is better to start with automation, or to at least incorporate certain automated unit operations, at an earlier stage—especially now that we are developing more sensors and controls.

Obviously, this is very hard to do in an academic research setting because the investment required may be too large. Therefore, at some point around the time that the process in question is being translated into industry, we need to think about how to incorporate automation and what the effect of automation will be on the product. We should also consider models where industry is more embedded or partnered with academic innovation centers to bring automation further upstream in the translation pipeline.

Q How far away is the goal of non-cleanroom GMP manufacturing with integrated process control for the cell therapy field?

KR: I do not think it is too far away. There are companies that are currently working toward that goal, and it is increasingly gaining traction both on the industry side of things but

also on the investment side. People are investing in these manufacturing equipment companies that may not require an ISO 7 clean room.

It can probably be done in either a lower-grade clean room or an entirely non-clean room setting, but the bottom line is that from a cost analysis standpoint, GMP cell therapy production represents a very large capital expenditure. Creating large clean room spaces, renting clean room spaces, or going to CDMOs for clean room spaces are all expensive options for small and medium-sized companies. Those smaller companies in particular are going to be looking into smaller footprint, fully closed systems that do not require high-grade clean rooms.

Q What are the next steps toward realizing the potential of fully automated cell processing systems?

KR: There is still a lack of biological knowledge on what can be controlled in the process in order to obtain the desired product quality. For instance, if I see that my iPSC-derived retinal progenitor cells are not giving me the measurements that they are supposed to after a week of manufacturing, how can I tweak things to control that situation? What can I add to or subtract from the culture that is going to drive it in the desired direction? That piece is not very well known in most of the cells types we work with today. There is still a large gap in understanding the fundamental biology of stem cell growth and differentiation.

On the technical side, we are still pretty far away from having sensor capabilities that are reliable, reproducible, and robust. We can measure a few things here and there, but a lot of the sensors on the market are not good for cell and gene therapy purposes. There is a lot that needs to be done in terms of sensor and measurement development, but also in terms of creating a small footprint for this technology, and achieving potency assays that can be integrated with bioreactors. Then, we need to bring in control system experts to get a better understanding of the process control algorithm. Once we know what we want to control and what we are trying to measure, we need to understand what the equation is, what are the kinetics, what is the dynamic control. There are examples of that being done successfully to some extent at the moment, but it is still a work in progress.

Q Can you elaborate on Georgia Tech's current activities and what you have planned for the future?

KR: Our primary focus in automation is entirely feedback-controlled, closed automation using control algorithms. We are in the process of trying to figure out what that algorithm and control system should look like for a specific cell type and for a specific application. For example, how do we integrate sensors dynamically with bioreactors? How do we integrate analytics? We are working towards answering those questions.

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BIOGRAPHY

KRISHNENDU (KRISH) ROY received his BTech from the Indian Institute of Technology (IIT), Kharagpur, his MSc from Boston University, and his PhD in Biomedical Engineering from Johns Hopkins University. After two years in the industry, Dr Roy joined the Biomedical Engineering Faculty at The University of Texas at Austin in 2002, eventually becoming Professor and Fellow of the Cockrell Chair in Engineering Excellence. In 2013 he moved to Georgia Tech, where he is now Regents Professor and the Robert A Milton Endowed Chair in Biomedical Engineering. He also serves as Director of three centers—the NSF Engineering Research Center (ERC) for Cell Manufacturing Technologies (CMaT), The Marcus Center for Therapeutic Cell Characterization and Manufacturing (MC3M), and the Center for ImmunoEngineering. Starting August 2023, Dr Roy will join Vanderbilt University as its next Dean of Engineering and an University Distinguished Professor in Biomedical Engineering, Chemical and Biomolecular Engineering, and Pathology, Microbiology, and Immunology. Dr Roy's research interests include scalable cell manufacturing, ImmunoEngineering, and controlled drug and vaccine delivery technologies, focusing on biomaterials. In recognition of his seminal contributions, Dr Roy has been elected Fellow of the American Institute for Medical and Biological Engineering (AIMBE), the Biomedical Engineering Society (BMES), and the Controlled Release Society (CRS). He has received numerous awards and honors, including the Industry Growth Award from Georgia Bio, the Young Investigator Awards from the Controlled Release Society (CRS) and The Society for Biomaterials (SFB), the NSF CAREER award, etc. He has also received the Best Teacher Award from the Biomedical Engineering Students at UT-Austin and the Best Advisor Award from Bioengineering students at Georgia Tech. Dr Roy serves on the Editorial Boards of the *Journal of Controlled Release*, the *Journal of Immunology and Regenerative Medicine*, and the *Journal of Advanced Biomanufacturing and Bioprocessing*. He is a member of the Forum on Regenerative Medicine of the National Academies of Science, Engineering, and Medicine and a board member of the Standards Coordinating Body for Regenerative Medicine.

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Quality by design planning in ATMPs: standards for manufacturers

Matthew Chorley, Global Vice President of Quality and Regulatory, BioIVT

The FDA's good tissue practices (GTPs) address the need to procure and process tissues and cellular starting materials in a manner that avoids transmission of a communicable disease. This FastFacts poster will explore what a cell therapy maker needs to consider when selecting a manufacturer to source cellular starting materials and related ancillary products that are GMP/GTP compliant. Careful consideration will allow for an efficient path for advanced therapy medicinal products (ATMPs) to move through the clinical pipeline and be approved for commercial use.

UNPACKING REGULATORY REQUIREMENTS

To maintain compliance and best practices, cell therapy firms must, at the very least, reference and include USP 1043, USP 1046, USP 1047, and CFR21 Parts 1271, 820, 210, or 211, depending on the stage and the nature of the product that they are dealing with.

Regulatory bodies recognize that cellular starting material can be highly variable due to process and donor variability. As a result, it is critical that starting material is collected, stored, shipped, and tested with a highly reproducible process.

IMPORTANCE OF A DEDICATED GTP/GMP SUPPLIER

Using a supplier who is incapable of manufacturing or supplying GTP/GMP-compliant cellular starting materials will cause a significant delay in the speed at which a therapy reaches market. As shown in **Figure 1**, GTPs and GMPs must be followed for cell and tissue-based advanced therapies at a minimum, during the middle clinical stage of the drug development pipeline (phase 2 at the latest is best practice) however, addressing this problem earlier is much better and aligned with best practice. If beginning the manufacturing process with a supplier who can only support research use only (RUO) materials, a cell therapy firm must then switch to a compliant supplier midstream.

COST OF CHANGING CRITICAL RAW MATERIAL SUPPLIERS IN CLINICAL DEVELOPMENT

Changing suppliers in phase 2 of clinical development would require:

- Qualification of a new GMP supplier
- Regression testing
- Additional product validation work
- Potentially repeating process performance qualification (PPQ)

These roadblocks increase cost and time to market, which, in turn, negatively affects patient welfare.

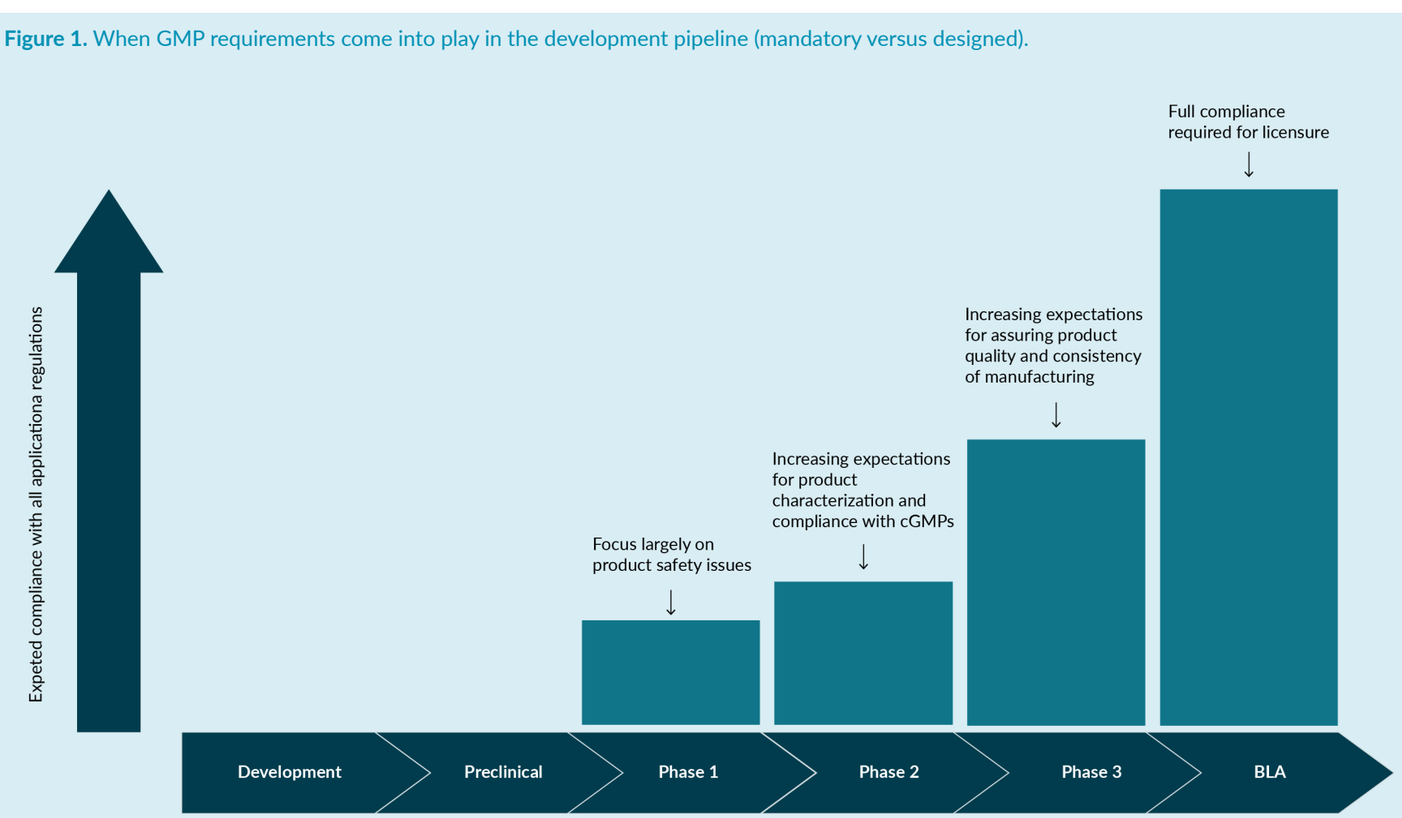
Additional considerations to make when choosing a starting material manufacturer

- It is difficult to change donor material late in the approval cycle due to product and process variability
- Dedicated and recallable donors should be one of the most important factors for selection of manufacturers
- A large network and donor base are necessary to ensure continuity of supply
- Primary and backup vendors are required to meet the demands of the growing market

CONCLUSION

The most critical and variable raw material in a cell-based therapy is the cell itself. To avoid delays, it is important for cell therapy firms to use suppliers of cellular raw materials that are compliant with GTP and GMP prior to mid- to late-stage clinical trials.

Figure 1. When GMP requirements come into play in the development pipeline (mandatory versus designed).



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In partnership with: BIOIVT

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

SPOTLIGHT

Scale-up and automation of iPSC-derived cell therapy manufacturing

Dhruv Sareen, Jonathan Rodriguez & Hojae Lee
Cedars-Sinai Biomanufacturing Center



VIEWPOINT

"We need automated solutions for taking the entire pipeline from start to finish. This end-to-end solution does not currently exist."

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On August 31, 2023, David McCall, Senior Editor, *Cell & Gene Therapy Insights*, spoke to Dhruv Sareen, Jonathan Rodriguez, and Hojae Lee from the Cedars-Sinai Biomanufacturing Center about challenges and progress in iPSC-derived cell therapy manufacturing. This article is based on that interview.

INTRODUCTION

The Cedars-Sinai Biomanufacturing Center (CBC) is a leading center of excellence for the manufacturing of induced pluripotent stem cell (iPSC)-based cell and gene therapies. Located in the center of Los Angeles, the center comprises 25,000 sq ft of state-of-the-art current good manufacturing practices (cGMP) biomanufacturing facility, including:

- 10,000 sq. ft of dedicated cell culture and process development (PD) laboratories,
- 11,000 sq. ft of cGMP cleanroom cell production and quality control (QC) laboratories, and
- 4,000 sq. ft of dedicated adjacent cell culture labs for incorporating hands-on stem cell training practical modules and education of the next generation of scientists specializing in biomanufacturing.

Notably, this center is integrated within a large health system that routinely performs clinical trials involving cell therapies.

The Biomanufacturing Center is capable of producing patient-specific (i.e., autologous) but also allogeneic stem cells for investigational new drug (IND)-enabling cell therapy studies, as well as providing these stem cells for research-based drug discovery programs for academic and clinical investigators. A robust academic and commercial project pipeline is currently underway to develop and manufacture clinical-grade cell therapy products suitable for investigational use in humans. In order to produce these cell therapies, the CBC has implemented an electronic quality management system (eQMS). QC is central to ensuring that any product manufactured at the CBC is of the required standard in terms of quality (e.g., safety, purity, identity, etc.) for human clinical trial use. The CBC now offers a panel of clinical-grade iPSC lines that can be non-exclusively licensed to investigators in industry and academia.

The Biomanufacturing Center's 55-plus staff members are focused on innovating around technological development and automation of the manufacturing of iPSCs, gene editing of cells, and providing those differentiated derivatives at scale. Other goals include providing quality testing and improved implementation of programs at Cedars-Sinai Medical Center, whilst also building a new training and workforce development program that is mindfully diverse, equitable, and inclusive as part of the California ecosystem – an initiative that is funded through the California Institute of Regenerative Medicine (CIRM). The Biomanufacturing Center also integrates with external manufacturing partners within California and across the nation to achieve a holistic approach to cell and gene therapy manufacturing as per cGMPs.

THE CURRENT STATE-OF-THE-ART IN iPSC-DERIVED CELL THERAPY MANUFACTURING

There are currently around 50 biotech and pharma companies funded to take iPSC therapy programs into the clinic, with the market growing at a rate of 8–10% annually

and predicted to be worth US$4–5 billion by 2026.

The common practice in iPSC material sourcing is to use either a skin biopsy or a simple blood draw from an adult or umbilical cord blood in order to isolate the starting material. Cedars-Sinai Medical Center is one of the few institutions that can take this blood in an FDA-compliant, donor-eligible manner [1]. Once the starting material is retrieved, there are various methods to reprogram the starting cells into iPSCs, including using Sendai virus, an mRNA-based virus, or DNA-based episomal plasmids. Cell reprogramming and isolation takes around 30 days, after which the cells are expanded to create GMP-compliant clinical-grade cell banks. The entire process typically takes around three months.

There are three major approaches to manufacturing iPSC-derived cell therapies. One is to take a healthy control donor iPSC line from either adult blood/skin or cord blood. A second approach, which the field is increasingly moving towards, is to create gene-edited 'hypoimmunogenic cells' or human leukocyte antigen (HLA)-matched iPSC lines. The hypoimmunogenic iPSC lines involve genetically engineering the surface of the iPSC to create a universal donor iPSC line. If one were to then derive a differentiated cell product from this universal donor iPSC line and transplant that product into a patient, it would not be rejected. This is currently a theoretical approach that has not yet been fully tested in humans.

Thirdly, scientists in Japan and Korea have adopted an approach that is used in the hematopoietic stem cell transplant field by creating a bank of the most common HLA haplotypes and deriving iPSC lines from donors who are homozygous for HLA-A, -B, and -DR loci. This results in approximately 10–20 iPSC lines that could match 60–70% of the population for a derived cell therapy. The CBC is also working on this approach in order to create an iPSC haplobank for the US population, which would require a greater number of HLA homozygous donor iPSC lines.

A key challenge currently facing the field is the standardization of QC testing. There are currently no defined guidelines for QC of iPSC manufacture. Commonly assessed critical quality attributes are purity, potency, identity, and safety of the final cell product. The majority of QC testing in the cell therapy clinical development pipeline is aimed at autologous methods, which require a lot of time to process—a fact that makes an allogeneic cell strategy the ultimate goal for every R&D pipeline in the industry.

AUTOMATION IN THE iPSC FIELD

Currently, using an autologous iPSC approach to gene edit and create a derived cell type for a single patient is not economically sustainable with traditional 'bricks and mortar' cGMP manufacturing. We need automated solutions for taking the entire pipeline from start to finish. This end-to-end solution does not currently exist. Automation is helping in defined steps of the process such as reprogramming, cell expansion, and differentiation, but these steps have yet to be integrated. Closed systems for cell therapy manufacturing are necessary to reduce the risk of contamination, reduce process time, eliminate extra steps, and increase cost-effectiveness. When scaling up iPSC manufacturing, automating the in-process control parameters using analytics can increase cost-effectiveness by eliminating batch failures.

Automation helps at Cedars-Sinai Medical Center during process creation—for example, to create a defined target iPSC in a 30–40-day process in order to make a muscle cell. Steps that can be automated at the Biomanufacturing Center include cell enrichment through magnetic sorting, cell isolation, and cell expansion. iPSC scale-up is enabled through robotics and automated incubators. Additionally, when growing iPSCs, the cells can spontaneously differentiate into unwanted

cell types. At Cedars-Sinai Medical Center, machine learning and AI-based approaches are employed to identify both wanted and unwanted cells. A laser-based approach can then be utilized to remove the unwanted differentiated cells.

One of the biggest challenges in the field is a lack of trained personnel and maintaining the workforce. Automation can be used to alleviate that problem, too, as it reduces potential contamination risk and process time when manufacturing cell therapies in GMP clean rooms. Automation also reduces operator fatigue and errors, as well as the need for extensive GMP training of manufacturing personnel.

REMAINING SCALABILITY CHALLENGES IN iPSC MANUFACTURING

The key challenge for iPSC manufacture relating to scalability is ensuring that at large-scale, one can obtain the same final product cell type consistently and with high quality that was produced at small-scale in the lab. This can be much more complicated than it may at first seem, because when scaling up, results can be unpredictable. For example, larger vessels have different fluid dynamics that can impact cell health and utilizing one may require a change in brand or material, either of which can impact cells health and kinetics in unexpected ways. There is also cell line-to-cell line variability in growth parameters, and if scaling up in a suspension culture bioreactor or adherent culture format, parameters may need to be reoptimized for each cell line. These parameters include the feeding regimen or the seeding density (an autologous approach will not require an undefined degree of scale-up, of course, necessitating a scale-out approach instead).

Another important remaining challenge when considering the scale-up of iPSC manufacturing is the cost of goods. Ensuring a sustainable supply chain can also pose issues, particularly pertaining to cases involving a single-source provider.

When iPSCs are pushed to scale and divide quickly, issues with genetic integrity can arise. Current literature has identified hotspots and mutations in putative tumor suppressor genes or oncogenes that require regular monitoring of cell banks during scale-up processes. The field does not yet have standardized defined QC assays that are clear and FDA-compliant to test the genetic integrity in the iPSC lines.

ENSURING SMOOTH & STREAMLINED SCALE-UP OF iPSC-DERIVED CELL THERAPY MANUFACTURING PROCESSES

At the CBC, the goal is to enable smooth tech transfer processes in both the internal and external client pipelines. The center implements a QbD approach early in the technology transfer phase. This transferring of manufacturing process and test methods involves drafting batch records early during the process development runs, comprising multiple training runs and pilot feasibility runs in non-GMP clean room labs. This is prior to GMP engineering runs where the batch production records are executed and improved upon, and to the drafting of a master production record for GMP qualification and clinical manufacturing runs in clean rooms. Again, automation is being implemented in certain steps in the pipeline. Critical quality attributes and critical process parameters are established early during the process development phase. A formal root cause analysis approach and a failure mode and effects analysis program are put into place to assess and avoid any issues with batch failures.

Over the next year, the CBC plans to build more data analytics and machine learning from each manufacturing campaign to help streamline an efficient manufacturing process. From a personnel standpoint, key goals include redefining the training matrix for both manufacturing and QC personnel.

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Anticipating such needs early on is critical to ensuring a smooth scale-up. Phase-appropriate approaches do hold value, but having a fit-for-purpose or qualified analytical method at an early stage is advantageous because it is not trivial to qualify and validate a test method.

A VISION FOR THE FUTURE

From an equipment and technology perspective, the vision is to build an integrated, automated iPSC manufacturing system that can go from a single vial of blood sample to a reprogrammed iPSC colony to an established iPSC line, expand it, create a cell bank, and then create a final differentiated cell product in an enclosed process. From a process technology perspective, the field needs to decide whether a universal hypoimmunogenic line, autologous iPSC line, or HLA-matched approach is best.

High-fidelity gene editing of iPSCs is also a necessity, as more and more companies and pipelines are using multiplex gene-edited iPSC lines to deliver their target payloads. To differentiate them from competitors, cell therapy developers have a particular gene of interest that is knocked in or out. Creating a uniform approach to high-fidelity gene editing would lead to fewer off-target effects when creating a genetically engineering iPSC line. From a regulatory standpoint, the question of whether such an approach requires full GMP-manufacturing or a 'GMP-like' process remains unclear for the cell therapy manufacturing community.

It is vital to gain a greater understanding of precisely what is needed by the US FDA in terms of iPSC manufacturing, analytical testing, and assay development. Genetic engineering of iPSCs will be a mainstay of future development in the cell therapy space, and establishing a portfolio of multiple assays will be necessary so that regulators feel comfortable that these therapies will be safe as they move into the clinic. If the gene editing process is not demonstrated to be stable and safe, then there will be no advancement of the next generation of cell therapies.

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CELL & GENE THERAPY INSIGHTS

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

SPOTLIGHT

INNOVATOR INSIGHT

An automated, closed system for rapid manufacturing of engineered T cells

Sophia Lollies & Ian Johnston

The recent successes of CAR T cell therapy in fighting hematologic malignancies have led to tremendous interest in the immunotherapeutic field, whilst the great potential of genetically modified T cells now expands into solid tumors and infectious diseases. However, the manufacturing processes used for engineering T cells consist of various complex procedures, are labor intensive, and represent some of the biggest challenges in this area. This article will describe the CliniMACS Prodigy® Platform and the new associated T Cell Transduction—Large Scale (TCT–LS) process, and present data from $CD8^+$ TCR-modified T-cell engineering in the setting of acute myeloid leukemia. Process details and differentiators will be shared, along with limitations and how to choose the right process type for your requirements.

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AUTOMATED T-CELL MANUFACTURING

When generating engineered T cells or any other engineered cell, automation reduces costs and lowers the need for multiple devices compared with manual operation. The benefits of automated cell manufacturing include reproducible and consistent results, reduced operator hands-on time, no extensive training of personnel required, and easily scalable production capacities. The CliniMACS Prodigy® Platform provides hands-off end-to-end automation in

a closed system, from R&D to commercial scale.

The CliniMACS Prodigy Platform has four components: the CliniMACS Prodigy Instrument, a fully automated platform for increased reproducibility and standardization; MACS® GMP Reagents optimized for a full end-to-end approach; closed tubing sets and consumables to ensure a safe environment; and the CliniMACS Prodigy software, consisting of a flexible configuration within a standardized process. Figure 1 illustrates the CliniMACS Prodigy Platform.

The CliniMACS system offers three different T-cell engineering workflows: T-cell transduction (TCT), T-cell engineering, and TCT–Large Scale (TCT–LS). The newest workflow, TCT–LS, will be the focus of this article.

TCT–LS WORKFLOW

The TCT–LS process workflow consists of cell selection, activation, transduction, expansion, harvest, and cell analysis outside of the platform. Reagents for the cell selection include CliniMACS CD4 and CD8. The activation of T cells is enabled by the MACS GMP T cell TransAct–Large Scale. Expansion takes place with TexMACS GMP Media and MACS GMP Cytokines such as IL-2, or IL-7 and IL-15 in combination. Harvest is performed in the CliniMACS Formulation solution. The tubing set is the TS 620, which has an enlarged cultivation chamber, allowing a final cell number of up to 20 billion T cells in 12 days (around 9 runs).

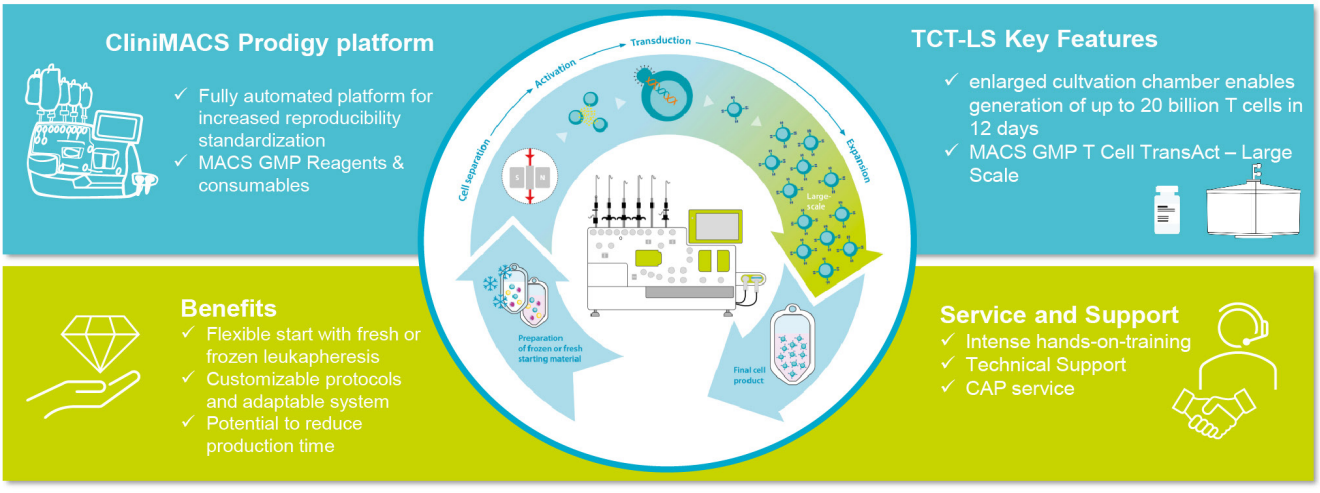
The TCT–LS system process allows fresh or frozen leukapheresis cell products as starting material for enrichment, with either choice showing no effect on expansion, cell count, or viability. This can be $\leq 10 \times 10^9$ white blood cells or $\leq 3 \times 10^9$ labeled cells (T cells), with acceptable volumes of 50–600 mL of fresh or 50–300 mL of frozen material. Seeding $2–4 \times 10^8$ enriched T cells is recommended as the starting material for culture.

The expected end product mean range is $1.46–2.05 \times 10^{10}$ T cells, with a mean transduction efficiency of 39.8%, though this is highly dependent on the construct, multiplicity of infection, and starting material. The CAR T cell end product mean is 6.5×10^9. The final volume after cell harvest is 100 mL (or custom up to 600 mL).

Customization of the cultivation phase is enabled via the Activity Matrix. Depending on needs different activities can be programmed, such as a medium bag exchange, medium exchange, feeds, or culture washes. The Activity Matrix auto-calculates and sorts the culture volume. The parameters of an

FIGURE 1

The CliniMACS Prodigy TCT–LS System overview.



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activity can easily be viewed, edited, added, and deleted.

CD8+ TCR MODIFIED T-CELL ENGINEERING IN ACUTE MYELOID LEUKEMIA

One application of the TCT–LS manufacturing process has been for CD8+ TCR-modified T-cell engineering in acute myeloid leukemia (AML). The therapeutic target in this case is the mutated nucleophosmin-1 (dNPM1), a common neoantigen in AML. A 4 bp frameshift insertion occurs in 30–35% of AMLs.

dNPM1-specific TCR was isolated from a healthy donor and cloned into the proprietary Miltenyi Biotec lentiviral backbone. This TCR recognizes the CLAVEEVSL peptide, which binds MHC II, in the context of HLA-A*02:01. TCR-peptide/MHC complex is dependent on CD8 for stabilization.

Due to this CD8-dependent activation and functionality of the modified T cells, only CD8+ T cells were isolated for manufacturing. This began with thawing the frozen apheresis and separating the CD8+ T cells. 2E8 CD8+ T cells were activated using MACS GMP TransAct–Large Scale to start the cultivation process. On day 1, a lentiviral transduction was performed. A manufacturing time of 8 days was selected for a rapid manufacturing window. In all development and qualification runs performed, the required cell dose was achieved within 8 days (Figure 2).

During manufacturing, in-process control (IPC) samples were taken to observe and enable decision-making. Quality control and immune monitoring of CAR T cell products are required. The number of cells present in the initial cell product must be checked to be adequate for manufacturing. Once cell separation is performed, the number of cells to be taken into culture must be quantified. After cell transduction, the transduction efficiency must be assessed. At the end of the manufacturing, CAR T cell quality, number, viability, and all other release criteria must be measured and analyzed.

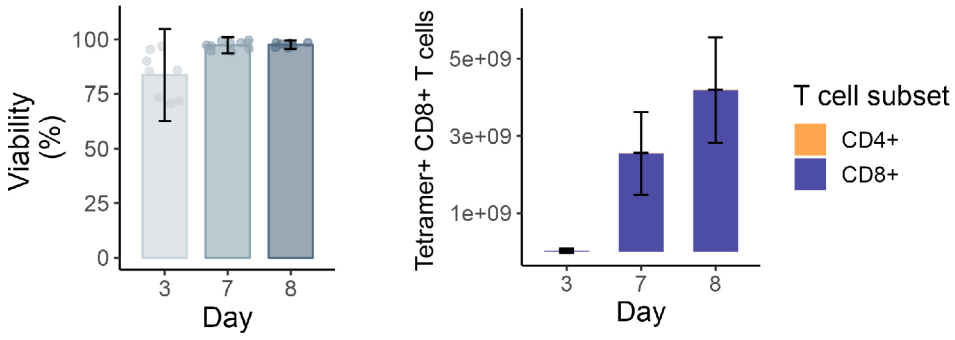
Tools are available for the analysis of the CAR T cells during manufacturing and patient monitoring. Several panels of ready-made recombinant antibodies are available in a dried format. These panels can be used to stain cell products or intermediate controls Express Modes, algorithm-based analysis tools, allow for fully automated acquisition and analysis of samples on the MACSQuant® Analyzer Platform.

TCR T-CELL MANUFACTURING PERFORMANCE

An analysis of the manufacturing of TCR T cells on the TCT–LS was performed. After enrichment, almost only CD8+ T cells

FIGURE 2

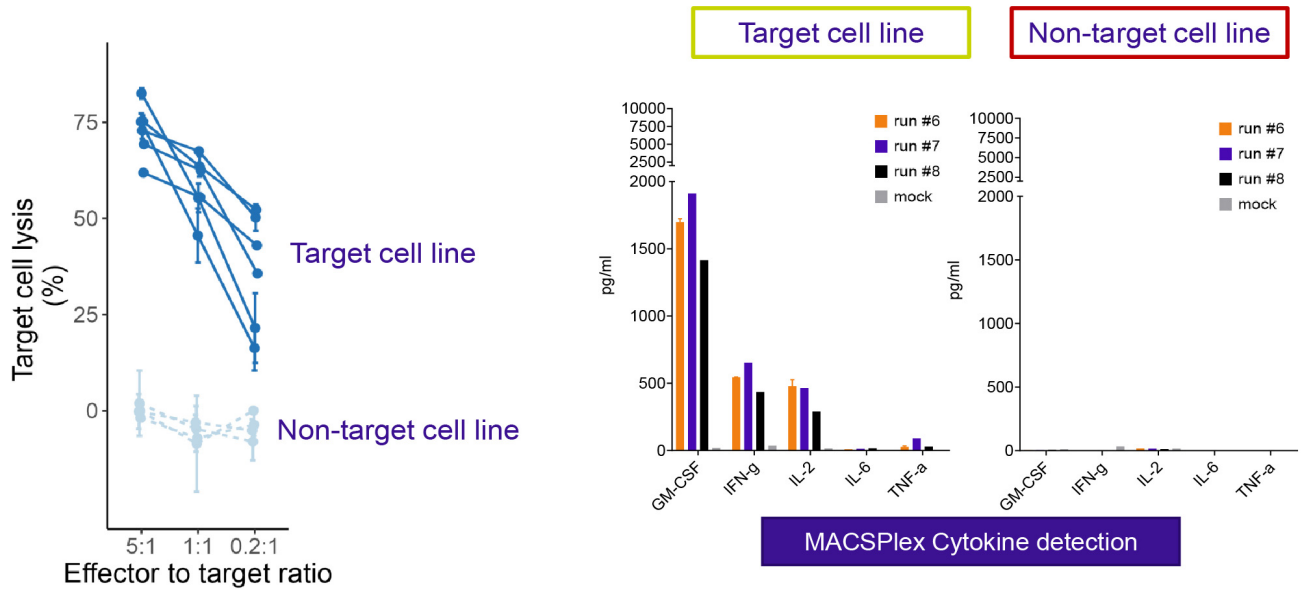
TCT–LS TCR T cell expansion performance.



Expansion for 8 days.

FIGURE 3

CliniMACS Prodigy TCT–LS functionality: 8-day manufacturing.



were present. In two runs, $CD4^+$ T cells were co-enriched.

T cells were then expanded with TexMACS supplemented with IL-7 and IL-15. Starting with frozen apheresis material, a mean of 8×10^9 $CD8^+$ T cells and 4×10^9 TCR-modified $CD8^+$ T cells was generated. Clinically relevant numbers of TCR^+ T cells could be generated within 8 days.

Robust TCR expression could be detected, which was modulated according to T-cell activation status. A key characteristic of TCRs is that the TCR expression changes during manufacturing. Different samples show variation in the levels of TCR expressed on T cells due to the activation status of the cells. CD3 and T-cell receptors are upregulated as the cells are activated, and as cells cool down a reduction in the level of TCR expression is seen. The integrated copy number stays constant within the whole cell population, with no variation in the genetic modification or loss of modified cells.

Functionality was tested by incubating the TCR-modified T cells with the target cell line, either expressing the wild-type or mutated form of NPM1. As shown in Figure 3, only in the presence of dNPM1 is a dose-related killing seen in this *in vitro* assay. No production of cytokines or killing is seen without the presence of the mutated peptide. Cytokines were measured using the MACSPlex cytokine detection system using multiplex beads, which allow measurements of all cytokines in one parallel assay.

Functionality was also tested in an *in vivo* model. Tumor cells were engrafted, which expressed dNPM1. The mice were randomized before injecting the TCR-modified T cells, and tumor growth was monitored every 3–4 days. After 17 days of tumor growth and 10 days after injecting the modified T cells, good control of the tumor growth was shown using the dNPM1-TCR-specific $CD8^+$ T cells.

CONCLUSION

TCR-modified T cells can be manufactured from a cryo-preserved apheresis product using a shortened manufacturing process of 8 days in a large-scale manufacturing system. This can generate an average of $4 \pm 1.4\times10^9$ TCR-modified T cells with a viability of over 97%. These cell products demonstrated target-specific cytotoxicity, both *in vitro* and *in vivo*.

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ASK THE AUTHORS


Sophia Lollies and **Ian Johnston** answer your questions on CAR T cell manufacturing using a closed, automated system.

Q You've shown data for autologous T-cell manufacturing. Is the system also suitable for allogeneic cell manufacturing of multiple doses?

SL: Yes. The system can electroporate. The CliniMACS Electroporator release can be added to the device and can perform a TCR knock-out or knock-in, with multiple complex gene engineering strategies enabled. However, with the TCT–LS system, we do not yet enable electroporation inside the software, so you would need a customized application to enable this. If larger amounts of cells (>600 mL), especially allogeneic T cells, are needed we also enable the connection of external culture devices to the prodigy with a customized application service.

Q For allogeneic manufacturing, sometimes multiple cell modifications are required. Are gene editing protocols supported?

IJ: Yes. The more complicated procedures with gene editing are possible on the smaller scale TS-520 system, where everything is well established. It is possible to perform quite complicated manufacturing using combinations of these technologies.

At the moment, the large-scale system does not support gene editing. Soon, it will be possible to use this larger-scale system plus the electroporator. This is already possible with special custom application procedures, but there is a ready-to-go system in development at the moment. There should not be any barriers to trying new and innovative processes on the instrument. This will enable early-stage research and translation into the clinic so that new protocols can be developed in a flexible way.

Q Does the process enable the use of frozen material?

SL: The device itself is not able to thaw frozen leukapheresis material. That must be done with a thawing device or in a water bath. However, the software enables a direct rapid dilution of the thawed leukapheresis by providing a sufficient amount of warm media to the application bag connected to the leukapheresis. The cells are in DMSO for a very short time and will be diluted directly, and then the process will start with cell washing right away.

Q Are there differences in performance or viability when using cryopreserved input material?

IJ: You may see a small amount of cell loss in the first few days of culture because the cells have been through a lot of stress. The separation procedure removes all those dead cells at the beginning to leave a relatively high viability for culture. Over the whole period of manufacturing, you will see no overall differences, but some small differences at the beginning of the process can be seen due to the cryogenic stress the cells have undergone at that early time point.

Looking at the manufacturing process as a whole, being able to start with a frozen sample gives you a lot more flexibility from the logistical side. At Miltenyi Biotec, we like to use a fresh product at the end point due to advantages in cell quality and performance, but you can also freeze down your end product for easier logistics.

Q How are QC samples collected?

SL: Connected to the tubing set, which is single use, there are sample pouches. Here, automatic IPC/QC samples can be collected at any time point. The user can tell the device to draw a sample and it will be fed into these pouches. When the sample is collected, we can analyze it for cellular composition or use the CAR detection panels.

Q How specific is the TCR you are working with? Does it show off-target toxicity?

IJ: We are fortunate that our target—dNPM1—is a neoantigen, meaning it is not present in any healthy cells and is only generated in AML. It is not yet understood exactly why this mutation occurs, but it is one of the classic mutations used to characterize AML when staging or categorizing patients. As it is a neoepitope and the TCR recognizes it came from a healthy donor, it is highly unlikely that this will have any off-target binding.

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We have characterized this together with our corporation partners with large peptide library sequence comparisons to show that there is essentially no off-target recognition. We see no background activity where you might expect a peptide to bind, so it seems to be a good target. We have not yet tested this in the clinic, which is our next goal moving forward.

BIOGRAPHIES

SOPHIA LOLLIES is a Global Product Manager for the clinical engineered cells portfolio at Miltenyi Biotec. Since 2021 she has been working with the R&D and Clinical Development teams to advance the development of CAR and TCR T cell therapy enabling tools, with a particular focus on CAR T cell immunotherapy. Prior to this, Sophia supported the development of clinical applications on the CliniMACS® Prodigy for industrial customers. Additionally, she gained relevant experience from Bayer AG in the field of global screening and automation, following the completion of her MSc in Life and Medical Sciences at the LIMES Institute in Bonn, Germany.

IAN JOHNSTON is responsible for the development of cutting-edge technologies to enable cell and gene therapies to be applied routinely in the clinic. Since joining Miltenyi Biotec in 1999, Dr Johnston has developed a number of reagents and procedures to improve the efficiency of genetic modification of primary cells such as human T and stem cells. A number of these procedures have been fully automated on a functionally closed cell processing platform—the CliniMACS® Prodigy Platform. In addition, Dr Johnston has led the development of numerous products for magnetic isolation of cells, molecules, organelles, and for use in the field of HIV research. He has been Principle Investigator on a number of European consortium grants focused on the genetic modification of stem cells, most recently Cell-PID and SCIDNET. His group is also investigating new targets for chimeric antigen receptors (CAR) to be used in T cell cancer immunotherapies. Prior to joining Miltenyi Biotec, he studied natural sciences at Cambridge University, where he also completed a PhD studying immunopathological mechanisms in the CNS. This was followed by postdoctoral research at Würzburg University with Professor Volker ter Meulen, working with recombinant measles virus systems, and supported by two fellowships from the Wellcome Trust and the Alexander von Humboldt Foundation.

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AUTHORSHIP & CONFLICT OF INTEREST

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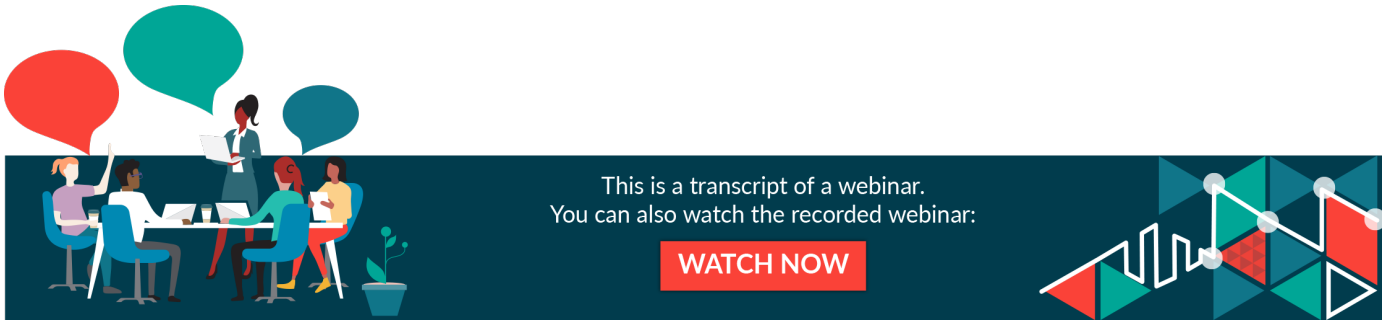

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CliniMACS Prodigy® T Cell Transduction – Large Scale

A leap towards commercial-scale CAR T cell manufacture

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CELL & GENE THERAPY INSIGHTS

LATEST ARTICLES:

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INNOVATOR INSIGHT

Regulatory considerations & validation strategies for mycoplasma testing for cell-based therapies

Mike Brewer

The chimeric antigen receptor (CAR)-T manufacturing and quality control workflow incorporates several components including cell isolation, cell selection, genetic modification, cell expansion, cell line authentication, identity and purity testing, potency testing, and microbiological testing. Regulatory guidance exists across the workflow; at the microbiological testing stage, mycoplasma detection is particularly important. Mycoplasma testing is typically performed after the cell expansion phase of the CAR-T manufacturing process, although additional testing points may be added based on process-specific risk assessment.

In this article, the current regulatory guidance regarding mycoplasma testing for cell-based therapeutic manufacturing will be discussed alongside the principles and performance of rapid, qPCR-based assays. An example of a validation study design will also be presented followed by a review and discussion of results obtained from a study executed per current regulatory expectations.

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CURRENT MYCOPLASMA TESTING REGULATORY GUIDANCE

In March 2022, the US Food and Drug Administration (FDA) released draft guidance to industry focusing on considerations for the development of chimeric antigen receptor (CAR)-T cell products. CAR-T products were defined as human gene therapy products in which the T cell specifically is genetically modified. Earlier FDA guidance for industry, released in January 2020, recommended that mycoplasma and adventitious agents release testing be performed on cell culture harvest material.

Typically, CAR-T products have a relatively short shelf-life and there is urgency in getting these drugs into patients. Therefore,

rapid mycoplasma testing, including PCR-based assays, is a necessity. Guidance recommends that mycoplasma testing is performed on the product at the manufacturing stage, and the assay is most likely to detect contamination after pooling of cultures for harvest, but prior to cell washing.

The guidance also recommends demonstrating that a test has adequate sensitivity and specificity during validation. The mycoplasma testing guidance from the Japanese Pharmaceuticals and Medical Devices Agency similarly reflects this guidance on where to test, stating that safety may be assured even if virus or mycoplasma testing is performed on crude bulks or intermediates, providing it is justified. Amplification of the virus or mycoplasma is not expected after the culture process.

Further regulatory guidance on testing for mycoplasma, released by the FDA for comments in March 2022, includes components that may be used in introducing the transgene—specifically, if a viral vector is being used to deliver the transgene into the CAR-T cell. It is recommended that testing should include microbiological testing during the manufacturing of the vector, such as sterility, mycoplasma, endotoxin, and adventitious agent assays, to ensure that the CAR-T drug product is not compromised.

Regarding pharmacopeial guidance for PCR-based mycoplasma testing, the European Pharmacopeia's guidance published in July 2007 states that nucleic acid amplification techniques (NAT) may be used as an alternative following suitable validation. The European Pharmacopeia also details the expectations around validation, which includes expectations around specificity in that a NAT or quantitative PCR (qPCR)-based assay for mycoplasma should be specific and inclusive to the species of mycoplasma, and should not detect bacterial species related genetically to mycoplasma. This guidance also addressed the sensitivity or limit of detection: ten colony forming units (CFU) or copy equivalent/mL as an alternative to the culture method, or 100 CFU or copy equivalent/mL as an alternative to the indicator cell culture method. Typically, both indicator cell culture and culture-based methods are used for mycoplasma testing for lot release. Additionally, the European Pharmacopeia guidance discusses robustness testing—deliberate variations, reagent volumes, incubation times, and sample storage conditions.

MYCOPLASMA DETECTION SYSTEM

The Applied Biosystems™ MycoSEQ™ Mycoplasma Detection Systemis a NAT-based alternative method designed for lot-release testing in a GMP environment, meeting or exceeding the European Pharmacopeia 2007 guidance. It includes an optimized sample preparation protocol for lot release testing applications and integrated analysis software with features to enable full 21 CFR part 11 compliance. Optional instrument validation (IQ/OQ) services are available, and the product is supported by a global network of experienced application scientists as well as regulatory experts.

The MycoSEQ Mycoplasma Detection System delivers a high level of confidence in the test results. It is sensitive, enabling validation at levels ≤10 CFU or 10 genome copies (GC)/mL. In line with specificity guidance, the assay does not detect any off-target organisms. It also uses objective multi-parameter results interpretation and has full workflow controls, including a discriminatory positive control. Following validation, regulatory filing, and review, end users receive regulatory acceptance to use MycoSEQ for lot release testing applications across multiple therapeutic modalities, including cell culture manufacturing, cellular therapy, and tissue therapy.

The Applied Biosystems™ MycoSEQ Plus Mycoplasma Detection Kit has recently been released. This kit employs a TaqMan probe-based chemistry to detect mycoplasma from

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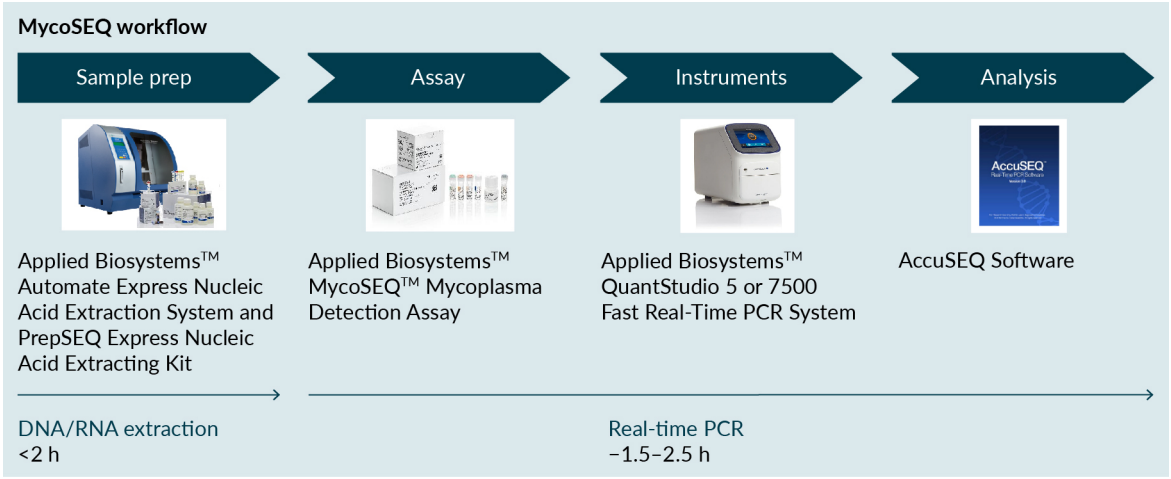
FIGURE 1

The MycoSEQ workflow: a complete sample-to-answer solution.



complex samples. For cell therapy and viral vector biotherapy manufacturers or testing service organizations that need to quickly and confidently ensure a product and materials are free from mycoplasma contamination, the MycoSEQ Plus Myscoplasma Detection Kit can deliver actionable results in less than a day.

The MycoSEQ workflow is a complete sample-to-answer solution from sample prep to analysis (Figure 1).

MycoSEQ's sensitivity is based on three components: background reduction and mycoplasma concentration, high-percentage nucleic acid recovery, and detection using the highest-sensitivity qPCR assay of 1–3 copies per reaction. Together, these components enable highest-sensitivity mycoplasma detection.

Sensitivity varies based on starting sample volume (Table 1). Different starting volumes do not enable detection at the same level of detection (LOD). If a 1 mL sample or a 10 mL sample is spiked with 100 CFU of mycoplasma or 100 GC of purified DNA, the final concentration will be 100 CFU/mL or 10 CFU/mL respectively.

NUCLEIC ACID EXTRACTION

Nucleic acids can be extracted either manually or automated via AutoMate Express. This fully automated extraction workflow uses prefilled cartridges that contain all the chemistry necessary to lyse the cells, bind the nucleic acid to the beads, wash away any impurities, and elute the DNA in a PCR-compatible solution. It takes an hour and 45 minutes to

TABLE 1

Assay sensitivity as a function of test sample volume.

Starting sample volume	Sample prep elution volume	Volume tested per qPCR reaction (1/10 of elution)	Assay sensitivity (GC/reaction)	Test sample equivalent volume PCR reaction	Sensitivity (CFU or GC/mL)
10 mL	100 µL	10 µL	10	1 mL	10
1 mL	100 µL	10 µL	10	100 µL	100
100 µL	100 µL	10 µL	10	10 µL	1000

process the workflow, and this can be executed on 13 samples in parallel.

MycoSEQ PERFORMANCE

The MycoSEQ Detection kit utilizes three independent parameters for results interpretation with the following acceptance criteria: cycle at threshold (C_t) ≤36; derivative value >0.08; and melting temperature (T_m) of 75–81°C. All three criteria must be met for a test sample to be positive for the presence of mycoplasma DNA. The AccuSEQ™ software enables automatic interpretation, analysis, and positive-negative calls.

An analysis of a ten-fold dilution series of purified *Mycoplasma arginini* DNA from 100,000 down to 1 GC/qPCR reaction is shown in Figure 2, alongside the melt analysis. Multi-component results analysis demonstrates a consistency that enables a high level of confidence in test results.

This discriminatory positive/extraction control DNA is made with a mycoplasma amplicon modified to have a T_m outside the range of standard mycoplasma amplicons, which allows an additional level of confirmation and confidence in a positive test result. The higher T_m allows discrimination between true mycoplasma and accidental contamination of the test sample with the positive control. This enables simple extraction control spiking of test samples, and the positive control can be used as a surrogate for mycoplasma DNA during method validation.

CASE STUDY: PRODUCTION BIOREACTOR CONTAMINATION INVESTIGATION

The following investigation took place on a contaminated production bioreactor. In this CHO cell production bioreactor, all operating parameters were within normal limits at harvest. The sample was submitted for the 28-day culture-based mycoplasma test, which gave positive results for the presence of mycoplasma.

Acholeplasma laidlawii was identified as the contaminant species by 16S rRNA sequencing. Equipment and facility decontamination, in addition to a root cause investigation, was initiated. Reserve in-process bioreactor samples were submitted for MycoSEQ qPCR testing, demonstrating how qPCR can be used to monitor not only during the production process itself, but also potentially at harvest.

The 14-day production bioreactor harvest was fed on a regular basis (Figure 3). On day

FIGURE 2

MycoSEQ assay readout and sensitivity.

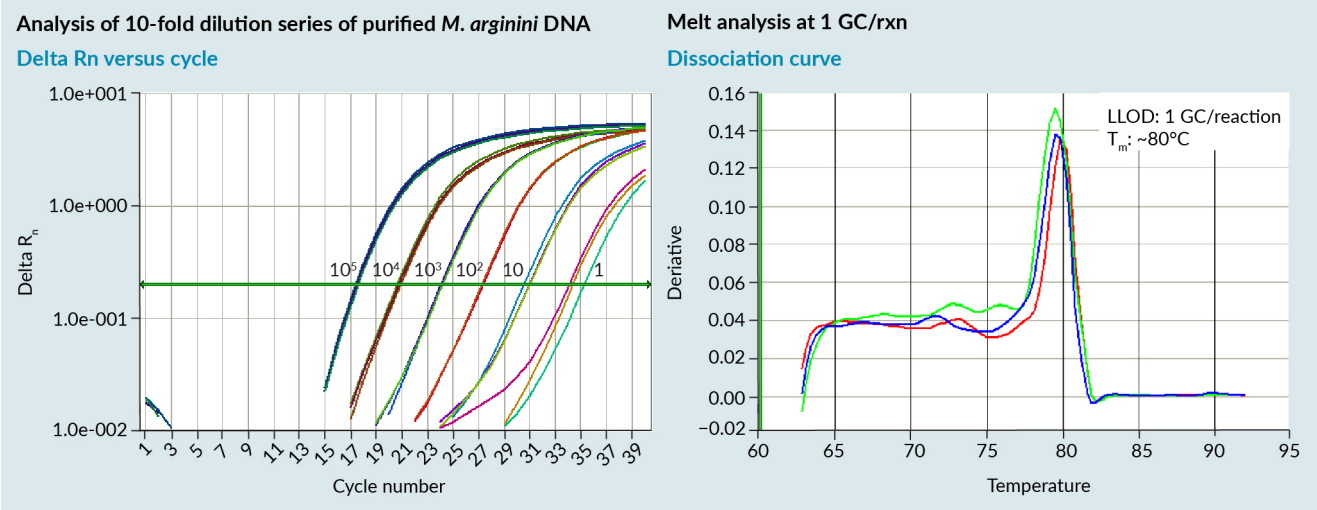


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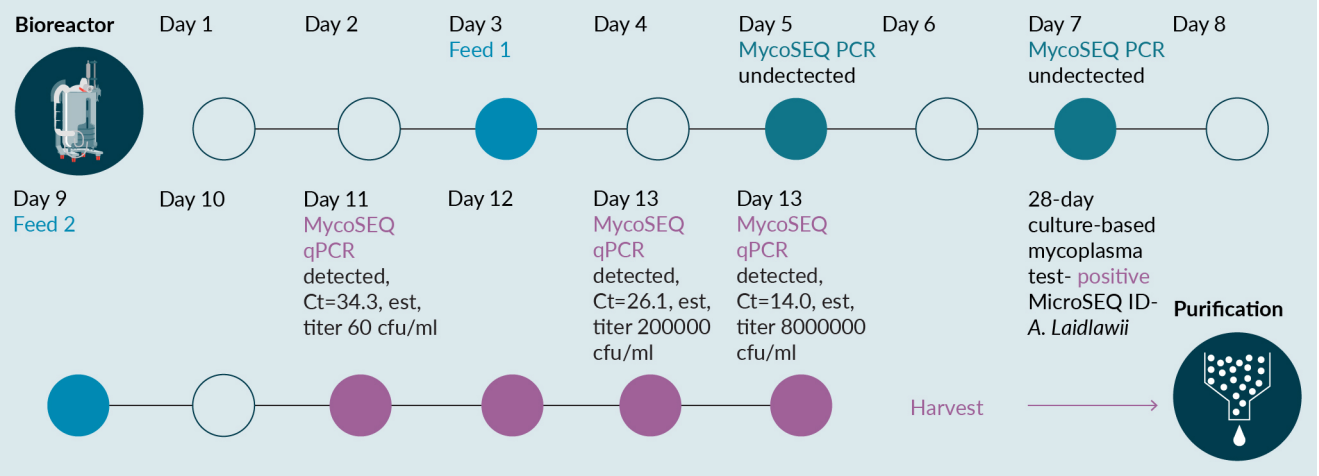
FIGURE 3

14-day production timeline and results.



three, the first feed was introduced. The reserve samples tested on days five and seven were negative for mycoplasma by qPCR. On day nine, a second feed was introduced into the process. qPCR analysis of the day 11 sample reserve showed the detection of mycoplasma, with a relatively high C_t of 34.3 and an estimated titer of 60 CFU/mL. The reserve sample from day 13 was also positive by qPCR, but the C_t was lower, indicating an increase in the level of mycoplasma DNA in the reactor. By C_t analysis, it was estimated that the harvest now contained about 20,000 CFU/mL. The following day, the C_t had dropped by 12, allowing an estimation that the level of mycoplasma DNA measured by MycoSEQ was up to 800,000 CFU/mL. The decreasing C_t values at day 13 and day 14 confirmed viability and provided an assessment of the growth rate of the contaminant species.

MycoSEQ qPCR is used to assess the impact of the contamination on downstream processes, and mycoplasma DNA was also detected in the purification process. The conclusion and the potential recommendations from this investigation were to use MycoSEQ qPCR pre-harvest testing as a conditional release test prior to downstream processing, and to also incorporate mycoplasma in-process testing using MycoSEQ.

CONSIDERATIONS FOR ANALYTICAL TESTING METHOD QUALIFICATION & VALIDATION

It is critically important to understand the analyte for qPCR NAT-based mycoplasma detection, specifically with DNA-based detection methods. Each mycoplasma cell has one circular chromosome (or 1 GC) per cell. The MycoSEQ assay is designed to target the 16S ribosomal RNA gene. All species of mycoplasma have at least one copy of this gene, with some containing two copies. Ideally, 1 GC=1 CFU, although this must be verified. If the ratio changes because the sample or stock has cells that contain DNA but do not grow and appear as a CFU, the sensitivity of a nucleic acid test may be misleading.

A key challenge that exists with LOD testing with live mycoplasma stocks is the importance of the GC:CFU ratio assessment to characterize those stocks. Mycoplasma can quickly reach log phase growth in liquid media. The stationary phase can be reached quickly, followed by the death phase or a decline in viability. When measured in the log or stationary phase, the GC:CFU ratio is close to 1:1, At the death phase, viable cells (CFU) decrease but GC remains stable. If CFU assessment (agar plating) is not

done at the appropriate time point, PCR- or qPCR-based LOD results could be misleading. Mycoplasma stocks used for LOD validation must be confirmed to have low GC:CFU ratio. qPCR sensitivity for DNA is not impacted by viability loss.

However, preparation of accurately quantitated mycoplasma stocks presents challenges, including but not limited to variability of GC:CFU ratio, and the accuracy of CFU titer due to clumping or aggregation of some mycoplasma species. Either of these issues can lead to misleading results during LOD validation. The stability of mycoplasma stocks has also been shown to be difficult to maintain. Degradation can lead to misleading results in validation if the titer is not verified immediately prior to use in LOD testing. The use of live mycoplasma in many testing laboratories presents an unacceptable risk. If the concentration of mycoplasma by centrifugation of the test sample is part of the sample preparation, recovery of mycoplasma from liquid samples, including cell cultures, has been demonstrated and is published widely.

A VALIDATION STUDY PLAN

Figure 4 shows an example validation study plan of a qPCR mycoplasma assay based on the ICH Guidance from Q2(R1) on Validation of Analytical Procedures, specifically as a limit test for mycoplasma DNA impurity. As per the ICH Guidance for validation of limit tests for impurities, there are two tests that must be performed: specificity and sensitivity/limit of detection.

The results from the Specificity Part 1 of a validation study executed at a contract laboratory, Mycosafe Diagnostics, in 2009 are shown in Table 2. In this experiment, purified DNA at 10,000 GC/qPCR reaction from a set of off-target species known to be genetically related to Mycoplasma, as well as common host cell species that are used in cell culture manufacturing, were assessed. Three individual qPCR reactions were performed for each species. All species gave a negative result for the detection of mycoplasma, and there was no interference in the assay.

The LOD results from the same external validation study are shown in Table 3.

In this case, for 10 mycoplasma species evaluated in the validation, all 24 qPCR reactions were positive. The C_t values were well below the positive-negative cutoff value of 36. Analysis of the 24 results showed that the values obtained were very consistent, which is an indication that the lowest limit of detection of the assay has not been reached.

FIGURE 4

An example validation study plan.

Specificity	LOD, part 1	LOD, part 2
Part 1: No detection of non-mycoplasma bacteria; data in DMF may be acceptable **Part 2:** No interference from customer sample matrix (also address in LOD, part 1)	**For each mycoplasma species included in the study:** • Six samples with 100 copies (10 GC/mL) purified mycoplasma DNA spiked into the lysate from 10 mL samples • Run four PCR reactions per extraction (24 total PCR reactions)	**This section may be optional depending on discussion with regulators. For each mycoplasma species included in the study:** • Six samples with 100 CFU (10 CFU/mL) live mycoplasma spiked directly into 10 mL samples • Run four PCR reactions per extraction (24 total PCR reactions)

CFU: Colony forming units ; DMF: Drug master file ; LOD: Level of detection ; PCR: Polymerase chain reaction.

TABLE 2

Specificity part 1: external validation results.

Species	PCR reaction number 1				PCR reaction number 2				PCR reaction number 3			
	C_t	T_m	D.V.	+/–	C_t	T_m	D.V.	+/–	C_t	T_m	D.V.	+/–
Hamster	Und.	71.7	0.018	–	38.9049	72.1	0.047	–	39.4103	71.7	0.032	–
Human	Und.	72.1	0.0094	–	Und.	72.1	0.027	–	Und.	72.1	0.0185	–
Mouse	39.8227	72.8	0.024	–	Und.	72.8	0.016	–	38.136	72.8	0.028	–
B. cereus	Und.	70.4	0.0095	–	Und.	72.4	0.017	–	Und.	72.8	0.021	–
B. subtilis	37.7234	75.2	0.0285	–	38.5207	74.9	0.0198	–	37.5753	75.2	0.0325	–
C. albicans	Und.	72.4	0.0113	–	Und.	65.5	0.0076	–	Und.	72.8	0.0088	–
Cl. perfringens	Und.	71.7	0.017	–	Und.	72.4	0.011	–	39.6925	72.4	0.031	–
E. coli	Und.	65.5	0.008	–	Und.	72.1	0.0172	–	Und.	72.1	0.0079	–
St. aureus	Und.	65.5	0.0095	–	39.2726	73.2	0.0385	–	Und.	65.5	0.009	–
St. epidermidis	Und.	72.8	0.0125	–	Und.	73.2	0.0123	–	Und.	73.2	0.015	–
Mc. luteus	39.9058	72.8	0.0305	–	39.0225	72.1	0.0285	–	Und.	72.4	0.015	–

TABLE 3

LOD results using purified DNA, 10 GC/mL using 10 mL test samples.

Mycoplasma species (type strain)	Total number tests/positive reaction	% positive	Mean C_t (n=24)	Standard deviation	Cell viability
A. laidlawii PG8^T	24/24	100	33.87	0.0625	1.8
M. arginini G230^T	24/24	100	30.90	0.99	3.2
M. fermentans PG18^T	24/24	100	32.21	1.68	5.2
M. hominis PG21^T	24/24	100	29.53	0.86	2.9
M. hyorhinis BTS7^T	24/24	100	29.22	0.85	2.9
M. orale CH19299^T	24/24	100	31.85	1.81	5.7
M. pneumoniae FH^T	24/24	100	33.03	0.73	2.2
M. salivarium PG20^T	24/24	100	31.14	0.87	2.8
M. synoviae WVU 1853^T	24/24	100	33.25	0.89	2.7
S. citri R8A2^T	24/24	100	32.79	1.65	5.0

C_t data can be compared from experiment to experiment and lab to lab, as demonstrated in Figure 5.

The upper two panels show the results from the MycoSafe Diagnostics validation study. 100% of the samples were positive, either with pure DNA or live Mycoplasma spikes. The C_t values were very similar between the two. Positive detection was obtained, and there was no indication of excess GC to mycoplasma cells. In the lower two panels, similar results are shown from a customer using mycoplasma stocks and DNA prepared by Bionique. 100% positive results were reported in both arms of the study. The C_t values were very similar and as expected based on qPCR analysis of purified *M. arginini* genomic DNA.

FIGURE 5

C_t data comparison.

10 mL test samples, spiked with *M. arginini* as purified DNA (GC/mL) or cells (CFU/mL)

Results from Mycosafe validation, 10 GC/mL:

Mycoplasma species (type strain)	Total number tests/ positive reactions	Percent positive	Mean C_t (n=24)
M. arginini G230	24/24	100	30.9

Results from Mycosafe validation, 10 GC/mL:

Mycoplasma species (type strain)	Total number tests/ positive reactions	Percent positive	Mean C_t (n=24)
M. arginini G230	4/4	100	32.8

Mycoplasma stocks and DNA prepared by Mycosafe

Results from customer validation, 8 GC/mL:

Mycoplasma species (type strain)	Total number tests/ positive reactions	Percent positive	Mean C_t (n=24)
M. arginini	24/24	100	31.1

Results from customer validation, 8 CFU/mL:

Mycoplasma species (type strain)	Total number tests/ positive reactions	Percent positive	Mean C_t (n=24)
M. arginini	24/24	100	31.5

Mycoplasma stocks and DNA prepared by Bionique

SUMMARY

The MycoSEQ Mycoplasma Detection System is designed for lot-release testing in a GMP environment, giving confidence in results. Over 40 licensed processes use the MycoSEQ System for lot release, following their successful validation, regulatory submission, and review. The MycoSEQ system is widely adopted across various regulatory jurisdictions and different therapeutic modalities.

Q&A

Mike Brewer

Q Can you go into more detail regarding sample volume requirements to achieve the LOD required by regulators?

MB: Typically, we recommend following the regulatory guidance of testing at the end of cell expansion—specifically, spent media with a defined amount of mammalian cells added to account for any potential cells associated with mycoplasmas. Additionally, if you want to test at the final dosage form, we recommend a smaller test sample volume with a lower level of detection (LOD).

Q When evaluating a rapid mycoplasma testing solution, what are the key features to look out for?

MB: Specificity, sensitivity, and support from the vendor. In terms of assay performance, you want the assay to be essentially agnostic to the mycoplasma species, in that the sensitivity will be approximately equal regardless of the species detected or tested in validation. These are all features that the MycoSEQ assay has. An experienced support team to provide training on the execution of the assay sample prep-optimization and data interpretation is ideal, alongside support with your qualification and validation study and subsequent regulatory submission and review.

Q Does the MycoSEQ system work on other qPCR platforms? What are the advantages of bringing this test in-house?

MB: It does work on other qPCR platforms, including any real-time PCR instrument that is able to read the SYBR Green fluorescent dye channel.

Bringing the assay analysis in-house provides a significant advantage in time to result. Typically, you want to quickly turn around test results following the end of the cell expansion. Ideally, you would test and get a negative result for mycoplasma before processing it through the washing, cell concentration, and final formulation.

Q When planning to bring a mycoplasma testing solution in-house, how long should we plan to have a solution implemented and validated?

MB: It depends on your timeline and how many people you want to dedicate to the project. If you are at the clinical trial stage, it is acceptable to use the assay as a qualified method. Typically, we can bring in Field Application Specialists to train you on the assay, the workflow, the interpretation of results, and confirm that your test sample performs with our typical sample prep for the chimeric antigen receptor (CAR)-T process. Then, you can do a quick qualification using purified mycoplasma DNA. Generally, we would qualify your test sample with two species, one high and one low sensitivity.

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The range is tiny: 1–3 GC/qPCR reaction at the lower limit of detection. We can qualify the assay with that. You do have to develop standard operating procedures in your lab for the operation and maintenance of the qPCR instrument. This can be done in 4–6 weeks, if you are on an aggressive timeline.

Q How do you validate the assay for use in GMP release testing? Is bridging to US Pharmacopeia (USP) compendial method required during the assay validation?

MB: Typically, our cell therapy customers do not run a comparability to USP 63 tests, primarily because for most of the CAR-T processes, the USP 63 tests cannot be applied. The guidance from regulators is clear that due to the short shelf lives of these products, rapid test methods are required for CAR-T processes. We have a long track record of successful validations and implementation and the vast majority of those did not use comparability testing.

We have additional data from our early adopters that did execute comparability studies that can be shared. If your individual reviewer does insist on comparability, we can guide you on a study designed to do that. The data we have so far indicates that qPCR is a more sensitive and accurate test.

BIOGRAPHY

MICHAEL BREWER is the Director, Global Principal Consultant, Regulatory for the BioProduction Group (BPG) at Thermo Fisher Scientific. In this role, Michael is responsible for providing global support to BioProduction customers and serving as the regulatory thought leader and expert across all technology areas within BPD. Prior to moving to this role, he led Pharma Analytics, a team responsible for development and commercialization of testing applications for microbiology, analytical sciences, and quality control. The products are fully integrated, solutions for bacterial and fungal identification, mycoplasma and viral detection, and host cell DNA and protein quantitation. Michael has over 30 years of experience in the biopharma industry, including, Scios, Synergen, and Amgen in a variety of roles including discovery research, analytical sciences, and quality control. Prior to joining Thermo Fisher Scientific, he led a group at Amgen that developed qualified, validated and implemented molecular methods for host cell DNA quantitation, contaminant (mycoplasma, virus and bacteria) detection, contaminant identification, strain typing, and genotypic verification of production cell lines. Additionally, his group supported regulatory submissions including IND, NDA, and CMC updates, regulatory inspections, NC/CAPA investigations, contamination investigations and remediation and developed regulatory strategy for implementation of new methods.

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AUTHORSHIP & CONFLICT OF INTEREST

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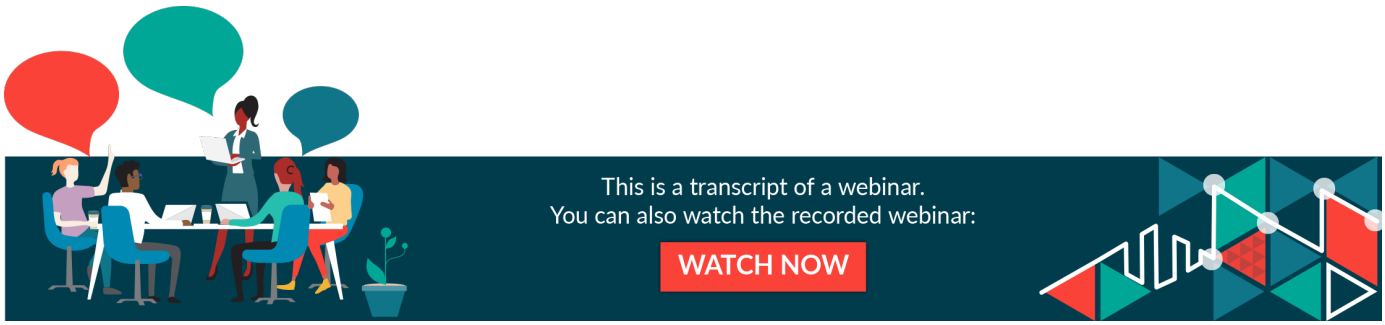

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applied biosystems

WEBINAR DIGEST

Improving biopharmaceutical quality and safety by implementing a microbial identification strategy in the workflow

Nico Chow, Field Application Specialist, Pharma Analytics Business Unit, Thermo Fisher Scientific

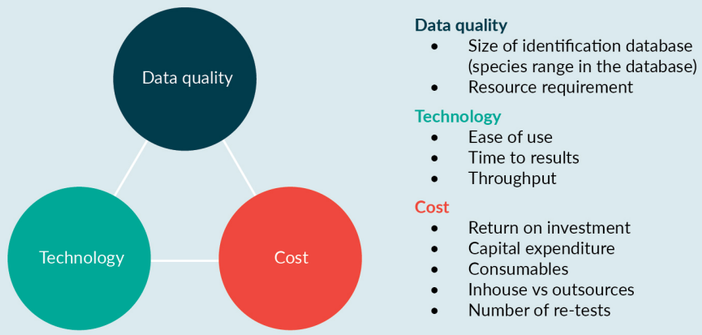
Following biopharmaceutical production, microorganisms present in drug products can compromise product quality and safety. These microorganisms can be difficult to remove and if present, may cause anaphylactic shock and even death in patients. Consequently, regulatory agencies require biopharmaceutical production facilities to implement an environmental monitoring program to enable drug product sterility. The Applied Biosystems MicroSEQ™ Rapid Microbial Identification System was designed to meet regulatory expectations for microbial control in cell and gene therapy workflows. This Webinar Digest summarizes the key aspects of the rationale and regulatory expectations for an effective environmental monitoring paradigm.

REGULATORY EXPECTATIONS REGARDING MICROBIAL CONTROL

Microbial control in biopharmaceutical production, such as in cell and gene therapy manufacturing, is a critical quality control step to ensure that drug products are free from viable microorganisms (e.g., bacteria or fungi). Microorganisms can release endotoxins which can harm patients and compromise product quality, including drug stability. It is crucial that both drug products and the facilities in which they are manufactured are free from harmful microorganisms.

Regulatory agencies require an effective environmental monitoring program in the bioproduction workflow that aligns with current good manufacturing practices (cGMP). At a minimum, the implemented program should enable the identification of microorganisms at the species level. It is also recommended that microorganisms be identified at the species level at frequent intervals to establish a valid, current database of contaminants present in the facility during processing.

Figure 1. Parameters for consideration when implementing a microbial identification system in a production workflow.



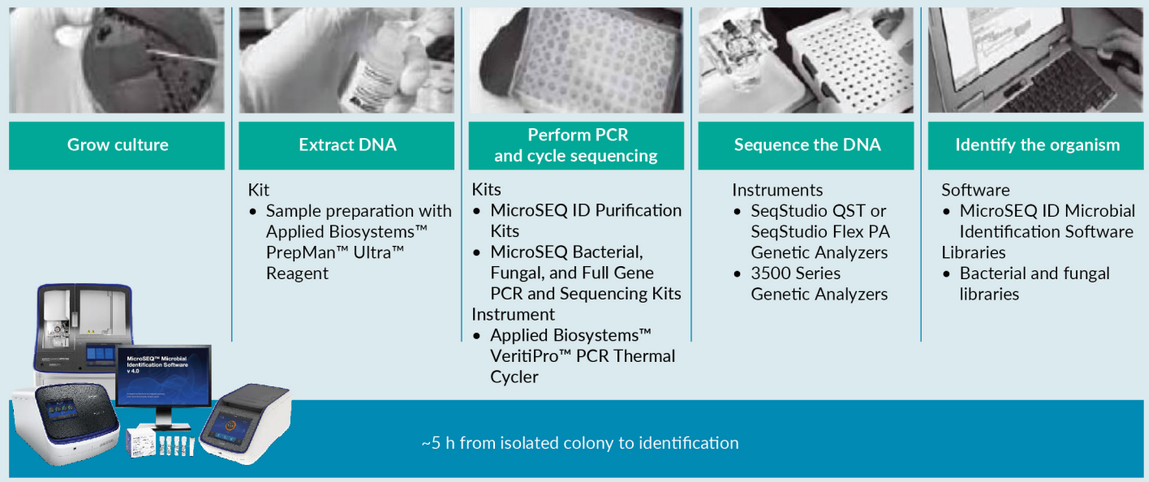
MICROBIAL IDENTIFICATION SYSTEM

Different microbial techniques are used to track possible contaminants in the biopharmaceutical production workflow; however, an effective environmental monitoring program requires alert levels for each microbial technique. Microbial identification (ID) is an important tool for identifying potential contaminants and enabling users to decide on the necessary actions for microbial control.

There are three main parameters to be considered when implementing a microbial identification system in a production workflow: data quality, technology, and cost **(Figure 1)**.

Although various techniques exist for microbial ID, genetic sequencing is considered to be the industry standard. The Applied Biosystems™ MicroSEQ™ Rapid Microbial ID System is a well-established genotypic system, designed to support the guidelines recommended by regulatory agencies worldwide. The MicroSEQ ID System is a complete end-to-end workflow solution that includes reagents for both bacterial and fungal identification, instrumentation, and software to enable progression from DNA extraction to automated data analysis of microbial species-level identification results. The system workflow has five easy steps, as shown in **Figure 2**, and generates results in less than 5 h.

Figure 2. The MicroSEQ rapid microbial ID System workflow.



In summary, accurate microbial identification during biopharmaceutical GMP production is crucial to ensure product and patient safety. Implementing an appropriate preventative and corrective program by utilizing genetic sequencing tools such as the MicroSEQ ID System limits any potential future failures in microbial control.

Watch the webinar here

Read the full article here

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FASTFACTS

Analyzing lentivirus particles using dPCR techniques

Samyuktha Shankar, Field Application Specialist, Pharma Analytics, Thermo Fisher Scientific

Lentiviral vectors (LVVs) are an active ingredient in biotherapeutics and must be tested for identity, purity, potency, safety, and stability according to regulatory guidelines. Therefore, reliable methods to characterize and quantify LVVs are critical to the success of many cell and gene therapies. This FastFacts poster explores an innovative digital PCR (dPCR) technology; a method for absolute quantification of nucleic acids, without standard curves.

WHAT IS dPCR?

dPCR is a method of quantifying nucleic acid targets by dividing the bulk PCR reaction into thousands of smaller, independent reactions. This method does not require a standard curve and is capable of providing absolute quantification of known genetic targets. Absolute quantification of a sample is achieved by counting positive reactions and applying Poisson statistics. Because no standard curve is required, this method is considered to offer greater precision and reproducibility when compared to other quantitative methods, even in high-background conditions.

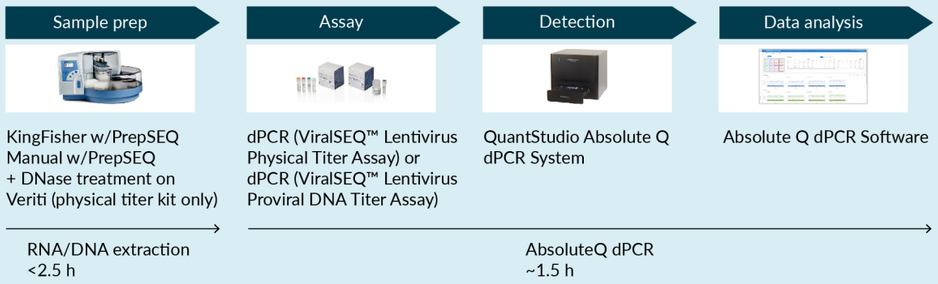
Limitations of existing PCR platforms include: significant wastage of sample; limited or inconsistent compartmentalization; tedious workflow (with multiple instruments and extensive human intervention required); long turnaround times (6+ h to generate a single data point); and the limited insight derived from endpoint analysis alone (leading to greater potential for a false positive result).

FLEXIBLE, INTEGRATED SOLUTION FROM PROCESS DEVELOPMENT TO GMP

The Applied Biosystems™ QuantStudio™ Absolute Q™ dPCR System addresses these limitations by providing:

- Reagent efficiency—<5% wasted reagent
- Consistency—20,000 consistent microreactions per array
- Easy-to-use workflow, equivalent to a qPCR system
- Fast time-to-results—90-minute run-time
- Confidence in data—automatic false positive rejection
- A single instrument
- Flexibility—4–16 samples per run
- Multiplexing—4-color capacity

Figure 1. EMA recommended standards.

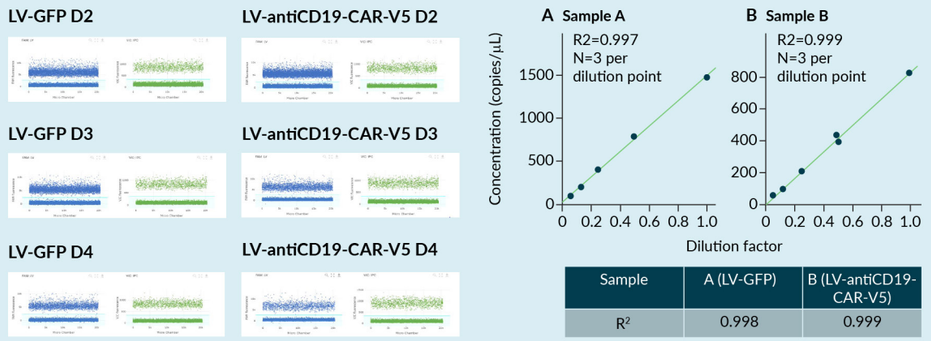


Two titer kits for quantitiation of LVVs can be used with the Absolute Q dPCR System: the ViralSEQ™ Lentivirus Physical Titer Assay and the ViralSEQ Lentivirus Proviral DNA Titer assay. These kits allow for the end-to-end workflow solution shown in **Figure 1**.

PHYSICAL TITER ANALYSIS

The analysis obtained for physical titer using the Absolute Q dPCR System is presented in **Figure 2**. The data on the left shows the fluorescence intensity (y-axis) of all microchambers (x-axis) for a given sample.

Figure 2. 'GMP-Like' versus GMP-Now™ plasmid DNA.



Sample	A (LV-GFP)	B (LV-antiCD19-CAR-V5)
R^2	0.998	0.999

Microchambers positive for the target on the lentiviral vector were FAM dye-labeled (shown in blue plot) and are above the blue threshold line; microchambers for the VIC dye-labeled internal positive control (shown in green plot) are also shown to be above the threshold. The data on the right illustrates the linearity of the dilution for the two samples, (A) LV-GFP and (B) LV-antiCD19-CAR-V5.

Figure 3 highlights the concentrations determined by the Absolute Q dPCR System and the adjusted concentrations in copies/mL and viral particles/mL. After accounting for the serial dilution factors, the concentrations were consistent, reflecting the high precision and accuracy of the assay. The total dilution factor was calculated using equation 1, then leveraged in equations 2 and 3 using the average dPCR concentration to determine the lentiviral stock concentration and the physical titer.

For research use only. Not for use in diagnostic procedures.

Figure 3. Calculating physical titer.

1 ▶ Total dilution factor=extraction dilution × DNase dilution × serial dilution × reaction dilution (e.g., for sample A, dilution D2 → total dilution factor=2 × 2 × 1250 × 5=2.5 × 10^4)

2 ▶ LV stock in copies/mL=average dPCR read × total dilution factor × 1000 µL/mL

3 ▶ LV physical titer in VP/mL=LV stock ÷ 2

LV Sample	Dilution	Average dPCR read copies/µL)	CV of replicates	Total dilution factor	LV stock (copies/mL)	LV physical titer (VP/mL)
A (LV-GFP)	D2	1479.29	0.97%	2.5×10^4	3.70×10^{10}	1.85×10^{10}
	D3	786.21	0.97%	5.0×10^4	3.93×10^{10}	1.97×10^{10}
	D4	395.99	1.60%	1.0×10^5	3.96×10^{10}	1.98×10^{10}
	D5	196.73	2.05%	2.0×10^5	3.93×10^{10}	1.97×10^{10}
	D6	96.57	1.76%	4.0×10^5	3.86×10^{10}	1.93×10^{10}
B (LV-anti CD19 –CAR-v5)	D2	822.21	0.43%	2.5×10^4	2.06×10^{10}	1.03×10^{10}
	D3	403.06	2.91%	5.0×10^4	2.02×10^{10}	1.01×10^{10}
	D4	202.61	3.33%	1.0×10^5	2.03×10^{10}	1.01×10^{10}
	D5	97.50	3.91%	2.0×10^5	1.95×10^9	9.75×10^{10}
	D6	50.56	1.82%	4.0×10^5	2.02×10^{10}	1.01×10^{10}
Positive control	NA	1368.66	4.47%	NA	NA	NA
Negative control	NA	0	NA	NA	NA	NA
No-template control	NA	0	NA	NA	NA	NA

CELL & GENE THERAPY INSIGHTS

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CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Utilizing an automation-on-demand analytical tool for cancer immunotherapy

Christoph Eberle

Novel experimental approaches aimed at weaponizing the immune system continue to dominate oncology research. As the immuno-oncology field evolves and demand grows, in-process development is essential to standardize recurring workflows pertaining to tumor sample handling in *in vivo* mouse oncology studies. Automation solution formats can be used as an on-demand solution for streamlining and simplifying processes. However, in order to effectively utilize fluorescence measurements, both understanding and control of pre-analytical variables need to be improved, as do the wash steps across different assay procedures. This article will explore a series of case studies involving the adoption of Laminar Wash™ technology, a recent refinement of the analytical toolbox associated with cancer immunotherapy.

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DEVELOPMENTS IN IMMUNOTHERAPY

Early immunotherapy research focused on blocking immune checkpoint inhibitor proteins such as PD-1, TIM-3, LAG-3, and CTLA-4 in order to elicit an anti-tumor response. This approach benefited some cancer patients. Simultaneous targeting of several of these checkpoint inhibitor proteins combined with other therapeutic interventions further improved response rates. However, tumors continued to evade recognition and destruction by the immune system. The development of bispecific antibodies designed to recognize two different epitopes at once represented a subsequent breakthrough. Initially, CD3 on T cells and tumor-associated antigens could be simultaneously bound by these constructs to trigger T cell killing and tumor elimination. Alternatively, simultaneously targeting two epitopes either on cancer cells or in the tumor microenvironment can block inhibitory pathways, reducing resistance to cancer therapy.

The adoptive transfer of expanded lymphocytes that already have the ability to fight their way into the tumor is a further, more passive approach to immunotherapy. The

lymphocytes are infused back into the same patient from whom they were isolated—a customized approach to cancer therapy that has also been adopted by developers of CAR T cell therapies.

These advances in immunotherapeutic modalities have been mirrored by innovation that enables preclinical drug development, including IND-enabling studies. Much of this activity has centered on mouse tumor models, where study animals are dosed and flow cytometry assays provide pharmacodynamic endpoint readouts. However, as innovation in the field has increased, analytical capabilities that were once limited to customizing flow cytometry panels have broadened to include protein detection platforms and other novel assay formats, technologies, and expertise.

TUMOR MICROENVIRONMENT CHARACTERIZATION & SINGLE-CELL SUSPENSION SAMPLE HANDLING

The tumor microenvironment is immunosuppressive, limiting the infiltration of naturally occurring tumor-infiltrating lymphocytes (TILs). The composition and tumor-killing ability of TILs can be modeled by immunotherapies, and response to such immunotherapy can be assessed in tumor-bearing mice by answering two essential questions:

1. Did the implanted tumors shrink, and if so, why?

2. Did the intertumoral lymphocyte composition change after dosing of a checkpoint inhibitor, for example?

Ideally, following immunotherapy, one may observe increased levels of total T cell infiltration, with a specific shift towards a larger population of $CD8^+$ cytotoxic T cells, as demonstrated in Figure 1 with an MC 38 model.

Immune cells infiltrating a growing tumor tend to become exhausted and therefore, unresponsive, due to chronic activation and expression of PD1 or CTLA-4 on T cells, and the presence of $FOXP3^+$ regulatory T cells giving rise to tumor immune tolerance. This immunological landscape (termed the 'cancer immunogram') is a highly-utilized atlas that assumes T cell activity is the predominant effector mechanism in tumor elimination. However, there are other lymphocyte subsets that can play a role, which is why a window is typically created into both the lymphoid and myeloid compartments when phenotyping the immune cell infiltration of a tumor by flow cytometry.

It is imperative to achieve the best possible tumor model sampling in terms of cell viability and recovery for downstream analysis using, for example, flow cytometry. Figure 2 shows a typical 'dose to data' workflow.

However, transitioning from tissue collection from mice through tissue preparation for processing into single-cell suspensions to immunostaining involves interdependent manual steps with many variables (Figure 3).

Depending on the specific tumor model, more or less debris and dead cells may be unwillingly carried throughout the single-cell suspension sample handling. This debris can non-specifically bind to and stain antibodies. If too much debris is retained in the sample compared to the fraction of relevant immune cells, it can hamper flow cytometry analysis. Results may be rendered inconclusive or even non-existent, due to this reduction in the percentage of relevant lymphocytes during sample preparation.

Automating the wash steps can streamline and simplify the TIL sample handling procedure. Figure 4 shows the results of utilizing the automated Curiox Laminar Wash™ platform to process samples. The data demonstrates that more viable lymphocytes in suspension samples are retained, regardless of the initial number of cells in the sample. Additionally, floating debris tends to be washed away over time, yielding cleaner samples than those obtained through the traditional centrifugation method.

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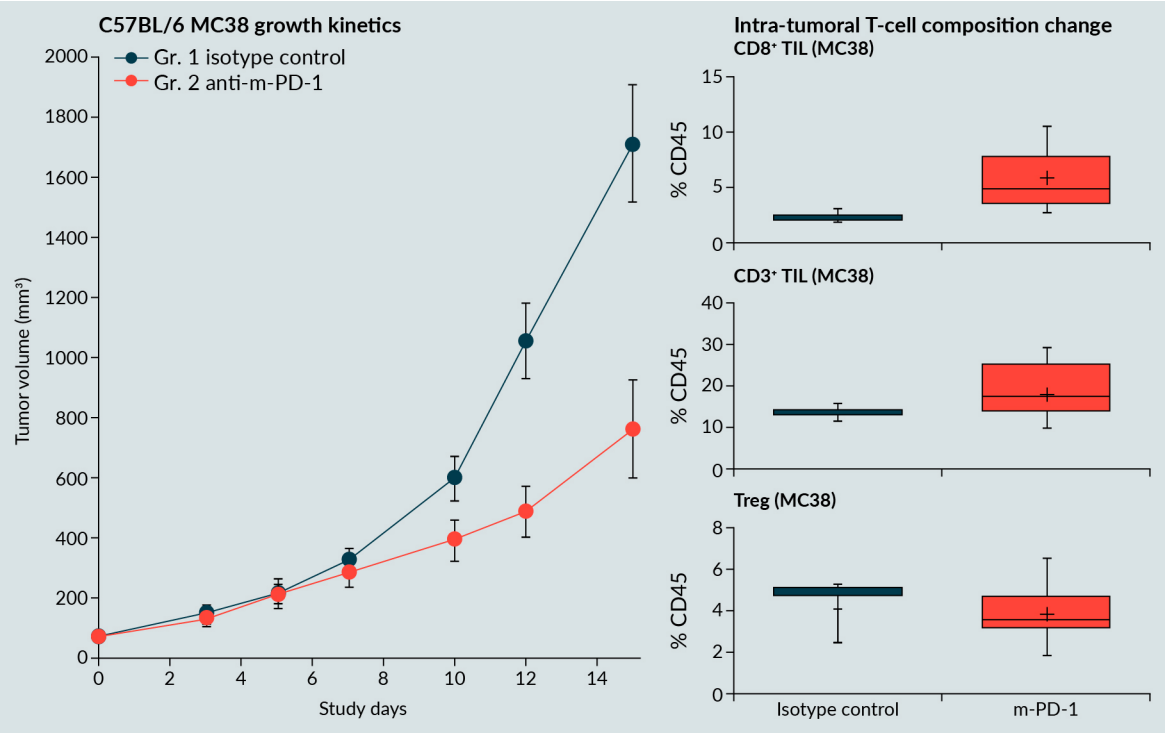
FIGURE 1

Murine T cells (CD3+) and subsets (CD8+) infiltrating MC38 tumors following immune checkpoint inhibitor (ICI) treatment.



Furthermore, traditional centrifugation results in mechanical stress, which has implications on cell physiology: the centrifugation process involves multiple cycles of pelleting manual supernatant removal, and subsequent pellet breakup before the crucial incubation steps during immunophenotyping. The Curiox Laminar Wash technology allows for reduced operator-to-operator variability when processing the same TIL samples for the same assay, as well as time and cost savings. In this example, these improvements allowed for faster generation of decision-enabling datasets for mouse tumor models.

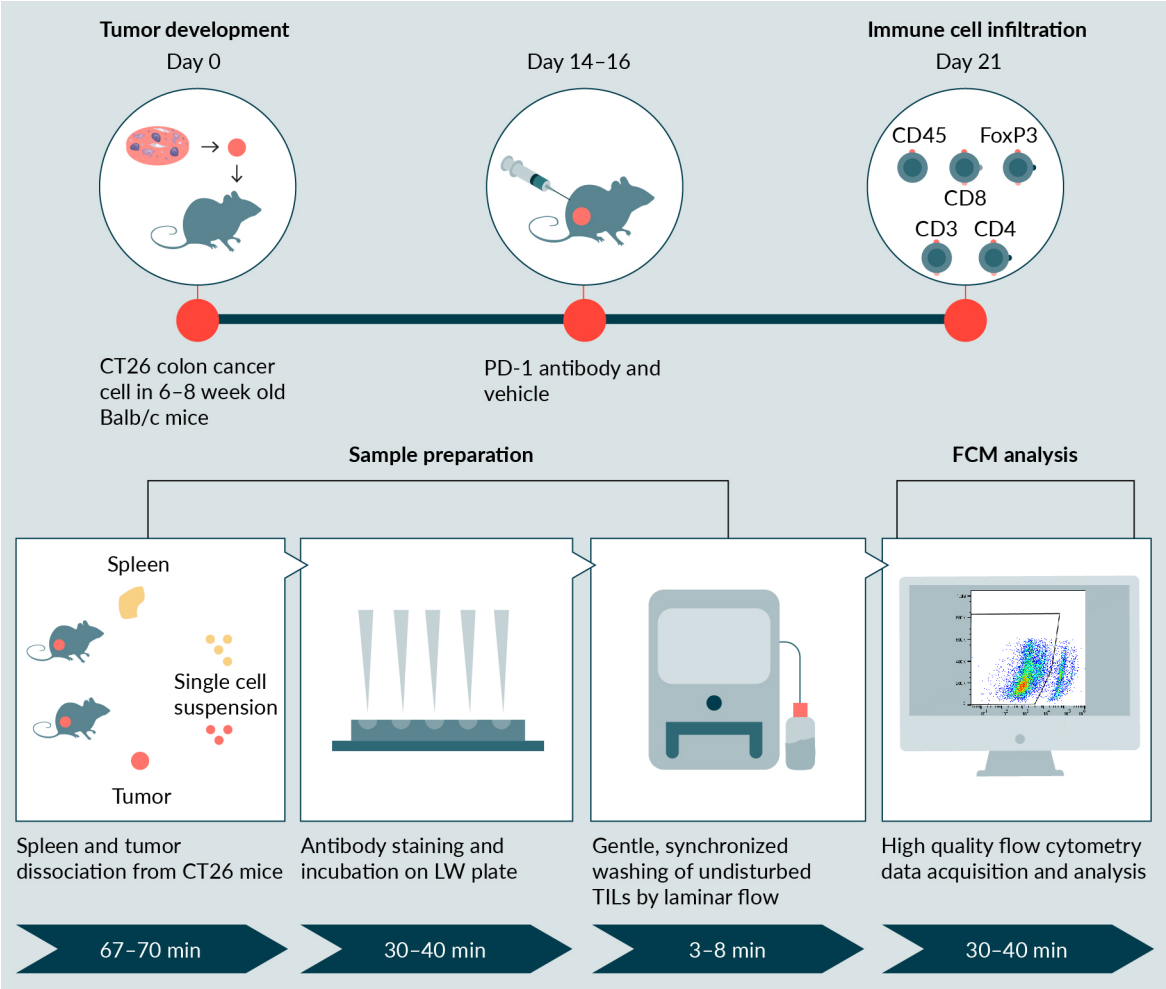
DEVELOPMENT OF ANIMAL MODELS TO IMPROVE PREDICTION OF EFFICACY FOR CELLULAR IMMUNOTHERAPIES

There have been major innovations around the development and refinement of humanized mouse models for testing safety and efficacy of experimental immunotherapies. Major components of the human immune system have been incorporated in mice, enabling improved translation into the clinic. This engraftment of human immune system components can be achieved in two ways; either human peripheral blood mononuclear cells (PBMC) are injected into severely immunodeficient mouse strains, or human hematopoietic stem cells (HSCs) are used to repopulate the bone marrow of myeloablated mice that are genetically predisposed to immunodeficiency through a lack of T cell, B cell and NK cell populations.

The first method rapidly yields a humanized mouse model with predominantly mature human T cells and CD4 and CD8 subsets suitable for short-term studies. A major drawback to this method is the development of graft-versus-host disease, which stems from the same mature human T cells attacking the major histocompatibility complex

FIGURE 2

Dose to data workflow.



Class I and II on tissues of the murine host. The second method of human HSC transplantation typically results in a fully mature, fully functional human immune system a few months post-engraftment.

There are also models for knock-in of a humanized checkpoint inhibitor pathway (e.g., PD1) into mice. This has the advantage of expressing only the human inhibitor protein in the context of an otherwise fully functional murine immune system. However, the translatability problem remains with all of these animal model options. Ultimately, the more closely a model resembles what can be observed in the clinic, the better. More recent trends in nonclinical model development aim at replacing animal models with alternative testing systems such as lab-on-a-chip, human organ-on-a-chip, organoids, and *in silico* methods.

BATCH ANALYSIS AS AN OPTION FOR PROCESSING TUMOR-INFILTRATING LYMPHOCYTE SAMPLES

From an operational point of view, it is beneficial to be able to analyze endpoint samples from various study terminations in a staggered fashion and without losing data quality, as compared to processing samples immediately upon study determination, which is the

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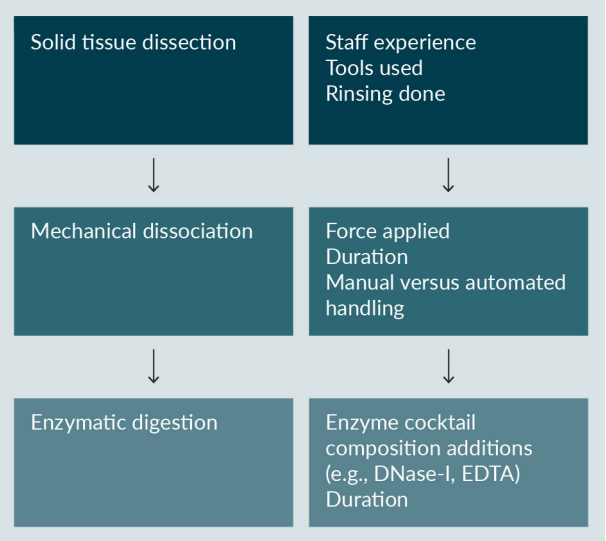
FIGURE 3

Tissue sample preparation variables.



procedure for immunophenotyping by flow cytometry. A study was conducted to explore a batch analysis option for TIL immunophenotyping, where all samples were processed by Laminar Wash technology.

Figure 5 and Figure 6 compare staining results, including viability and recovery, from a simultaneous TIL processing experiment in which digested tumor tissues were processed fresh before undergoing a single freeze-thaw cycle. MC38 tumor tissues were collected and digested before single cell suspensions were prepared. These suspensions were then aliquoted into two sets. One set was phenotyped on the same day, and the second set was frozen, stored at -80°C and after 7 days, thawed. The recovered thawed lymphocytes were then processed using the same methods as the fresh tumor tissues—centrifugation and Laminar Wash technique. It was found that, after undergoing one freeze–thaw cycle, Laminar Wash-processed MC38 TIL samples reproducibly exhibit higher retention rates of lymphocytes.

The surface immunostaining results, using a simple five-color panel (Figure 6) are the same for both the fresh and the recovered TIL samples processed using the Laminar Wash technique. The differences in recovery rates between centrifuge and non-centrifuge samples become apparent upon analysis of the actual cell numbers for all lymphocyte populations. Here, it is evident that Laminar Wash process samples can minimize cell loss across all TIL populations.

The data shown in Figure 7 emphasizes that Laminar Wash process samples can minimize cell loss across the lymphocyte populations.

These initial results support the idea that the Laminar Wash technique could be a

FIGURE 4

Simultaneous processing by traditional centrifugation and Laminar Wash platform.

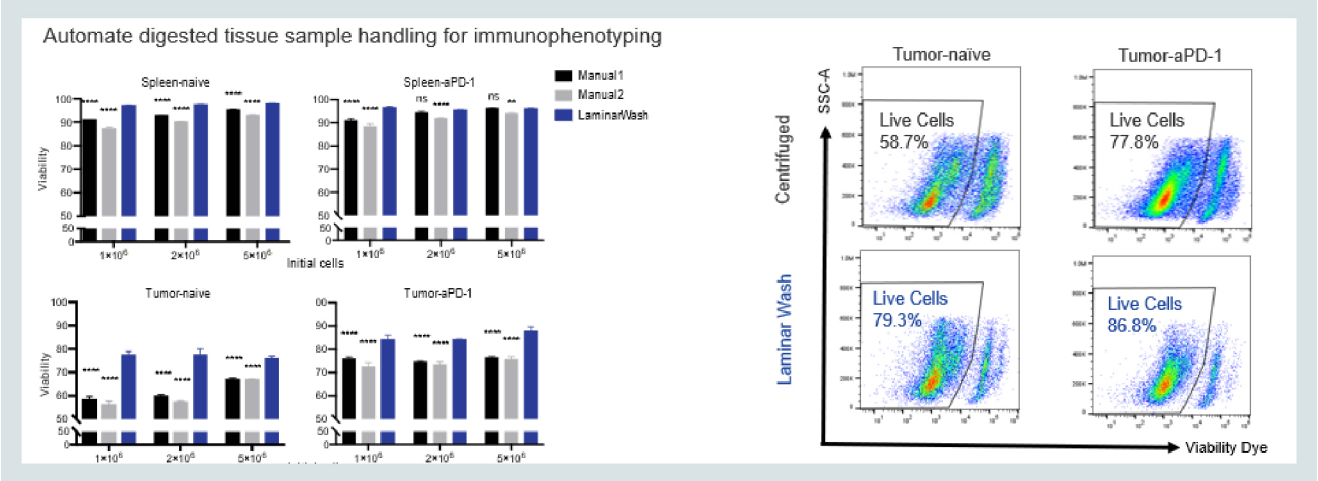
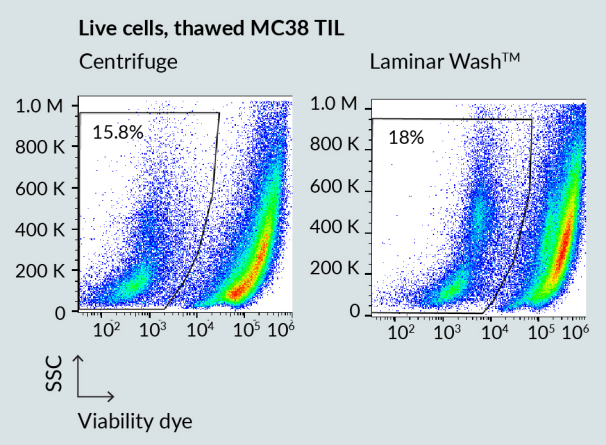


FIGURE 5

Viability staining results from thawed MC38 TIL samples processed by Laminar Washing and centrifugation simultaneously. Viability and recovery are equivalent to the freshly prepared MC38 TIL sample set only when processed by Laminar Washing (dot plots not depicted).



method of choice to process tissue samples in standardized preclinical workflows. More specifically, this technique could standardize endpoint readouts done for a certain type of study at one or several sites. However, there are a few caveats to note. Firstly, this assessment was conducted with a simplified color panel using surface markers that can only identify major immune cell populations. Even if this is a viable path forward, there are some limitations, including those that result from the available infrastructure at any given site or network of sites. Capital expenditure is essential to attain the required instrument.

In addition, an evaluation of each flow cytometry staining panel in a project would be needed to understand potential pitfalls. These pitfalls could come from individual marker selection, panel design, reproducibility of surface staining and intracellular staining results, and reproducibility of staining artifacts. It is also unknown whether the reduced loss of cell samples when processing samples by Laminar Wash technique guarantees the detection of rare events. Long-term and short-term stability needs to be established, and further research must be conducted to determine whether this approach can be applied solely to common specimens such as PBMC, or if it could beyond TIL samples to other digestive tissues (e.g., heart, kidney, liver).

DETECTING IMMUNE CELL-MEDIATED ELIMINATION OF TUMOR CELLS WITH ASSAYS

Antibody-drug conjugates (ADCs) are designed to specifically target cancer cells

FIGURE 6

After one freeze thaw cycle, Laminar Wash (LW) processed MC38 TIL samples show reproducibility.

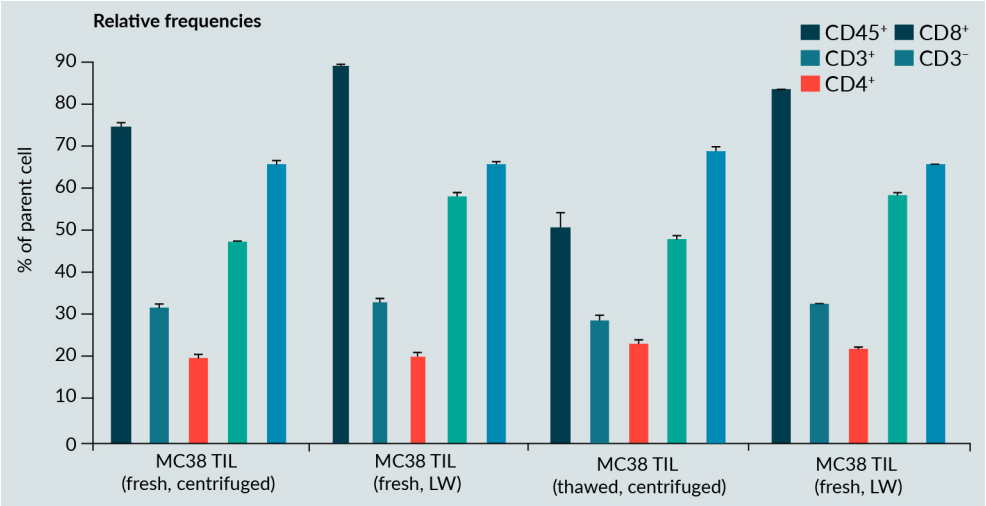


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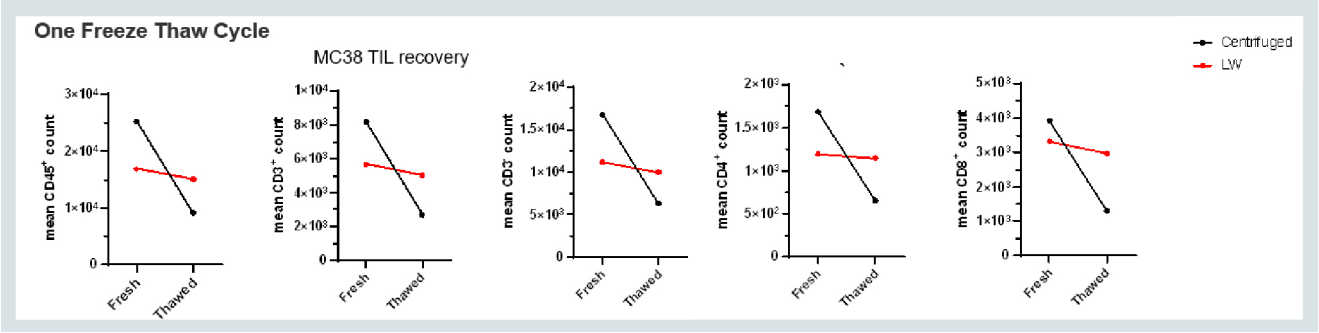
FIGURE 7

MC38 recovery after one freeze thaw cycle.

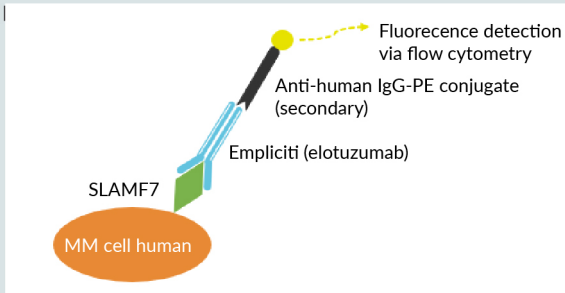


whilst sparing healthy surrounding cells. The antibody component used in ADCs are predominantly immunoglobulin G antibodies, which consist of four subtypes; IgG 1, 2, 3, and 4. IgG 1 is the most abundant in serum and by high-affinity binding to Fc receptors on effector cells such as NK cells. These complexes can trigger immune mechanisms such as antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent phagocytosis, or complement-dependent cytotoxicity.

The immune cells primarily responsible for tumor elimination are $CD8^+$ cytotoxic T cells and NK cells. It is essential to establish a known assay format that allows immunodetection of IgG-based antibodies targeting human multiple myeloma (MM) cells. Figure 8 provides an example assay format. As a positive control for this antibody binding and subsequent detection, a biofluorescently-labeled secondary antibody can be used. Elotuzumab (Empliciti®) is used for this purpose. Empliciti can engage the signaling lymphocytic activation molecule family 7 (SLAMF 7) expressed on myeloma cells while triggering NK cells through Fc receptor interaction to eliminate the cancer cells via ADCC. Empliciti can also trigger NK cells directly by binding to SLAMF 7 on NK cells.

FIGURE 8

Immunodetection of elotuzumab bound to human myeloma cell line.



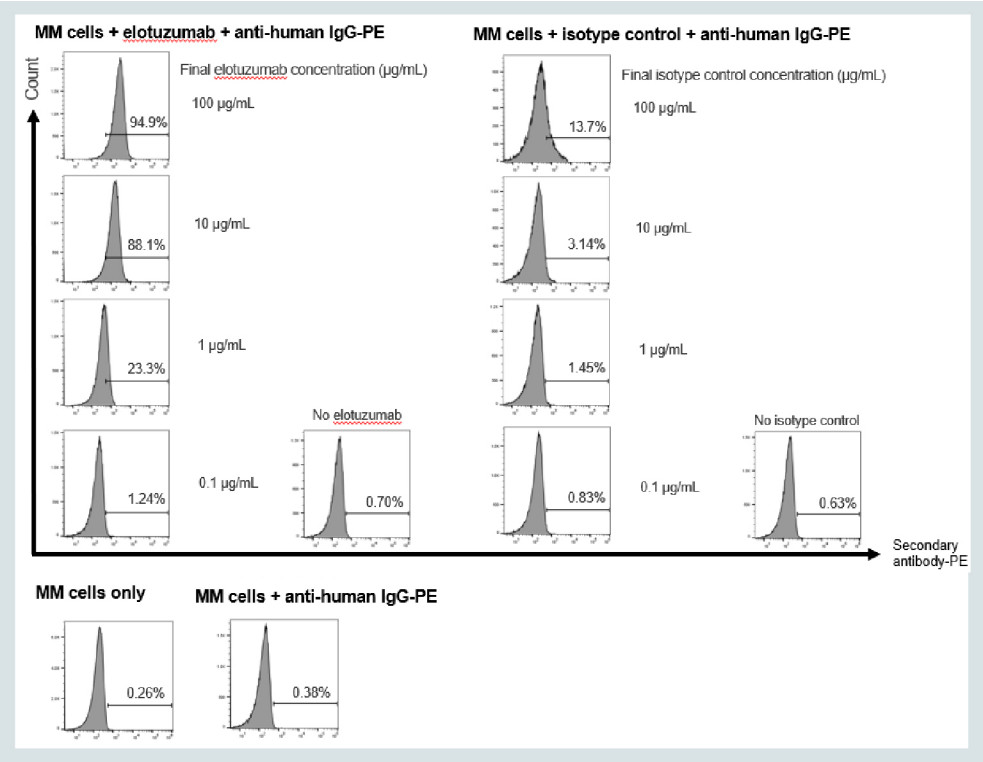
However, unexpectedly, a high background was observed with the myeloma cells and the secondary antibody conjugate. In addition, there was a lack of actual binding specificity with all three components of the assay—the myeloma cells, Empliciti, and the secondary detection antibody. Time was limited, as taking too long to troubleshoot could have derailed the entire project. Therefore, the Laminar Wash platform was used to quickly establish the binding assay. As shown in Figure 9, with an optimized protocol, ADCC dose-response curves derived from human multiple myeloma cell lines were quickly and easily generated.

TIME: A CRUCIAL FACTOR IN ASSAY DEVELOPMENT

Time is the most important asset in drug development. Charles River Laboratories is providing the industry with a strategic advantage when it comes to time, from identifying a

FIGURE 9

Binding assay protocol development using Laminar Wash platform.



pipeline of promising therapeutic candidates, to the testing phases in animals and *in vitro* models, to clinical studies in patient cohorts. Losing time could result in failure to secure market access with a new modality.

Therefore, factoring in time is at the forefront of pipeline strategy planning. For the still-nascent cell and gene therapy field, there are no established methods, let alone standardized ones. Often, it is unknown what kind of method or methods to develop but nevertheless, choosing the appropriate option is a key part of running a successful program.

For this new class of advanced therapies, analytical method development should be a consideration from the beginning in order to map out a comprehensive path for a product candidate into the clinic. If automation is available as a plugin tool with the potential to fit in any drug development stage, the workflow required for analytical data output could be accelerated while productivity is improved and operational costs reduced. Automated solutions such as Laminar Wash technology are ideal in that they can be deployed across multiple assay formats and procedures, are scalable, and are operable in various regulatory environments without involving the requirement to add different supporting instruments for each type of assay.

AUTOMATED TOOLS TO INVESTIGATE CELL SIGNALING MECHANISMS BY FLOW CYTOMETRY

Phospho-flow cytometry presents a challenge as it is a specialized application for measuring the phosphorylation status of proteins that modulate cell signaling pathways and cell signaling responses. Laminar Wash technology was applied to phosphoproteins because they

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are wash-intensive. In addition, there is a relevance to cancer research in understanding in which immune cells activation and downstream signaling events occur by phosphorylation and de-phosphorylation. Additionally, the detection of a signaling protein from the class of histone deacetylase (specifically, phosphorylated histone deacetylase 4) was measured.

In side-by-side processing of freshly isolated human PBMC, traditional centrifugation and the Laminar Wash technique were compared. The Direct Reading Grid, along with the 96-well plate that typically fits onto the HT2000 platform, were incorporated. The Direct Reading Grid is an accessory tool that can be placed directly on the Curiox Laminar Wash plate at the final resuspension step, prior to read-out at the autosampler unit of the flow cytometry system. This setup is beneficial because it circumvents the transfer of already-stained Laminar Washed cells to a separate reading plate, avoiding both losses and newly introduced variabilities.

With the Direct Reading Grid, the fluorescence intensities of the phosphorylated histone deacetylase 4 in resting cells were equivalent for both mean fluorescence intensity (MFI) and geometric mean fluorescence intensity (gMFI) values in both centrifuged and non-centrifuge samples run in technical triplicates. This basic Laminar Wash protocol was then further optimized with varying stimulation conditions in conjunction with T cell marker staining.

Ideally, these optimized settings will be used as an assay template that can easily be applied directly, or adapted with multiple multiplexed phospho-flow panels without sacrificing robustness.

THE FUTURE OF CENTRIFUGE WASHING

Moving forward, every assay developer who handles suspension samples should give thought to the optimal velocity for processing single cells. While centrifuges are currently the default setup in laboratories, drug development scientists should explore alternate ways of sample handling. No matter the degree of complexity for a particular procedure, the cell suspension wash steps tend to be comparable across various protocols. As a result, the field may collectively move away from traditional centrifugation and towards a gentler method that can wash and consistently recover cells without losing viability, and without potentially altering those cells during assaying.

With the examples highlighted here, from standard TIL immunophenotyping to phospho-flow cytometry, the adoption of Laminar Wash technology has proven to be a complementary solution, allowing for greater precision, accuracy, and sensitivity when measuring single cells.

BIOGRAPHY

CHRISTOPH EBERLE is a Principal Scientist III who has established and overseen the flow cytometry core at the Charles River Discovery site in Worcester, MA since he joined in 2017. He directs immunophenotyping of tissue samples collected from various mouse models to include T cell isolations for adoptive transfers and immunoassay development on various protein detection platforms.

AFFILIATION

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BIOLOGY AT ITS BEST

AUTHORSHIP & CONFLICT OF INTEREST

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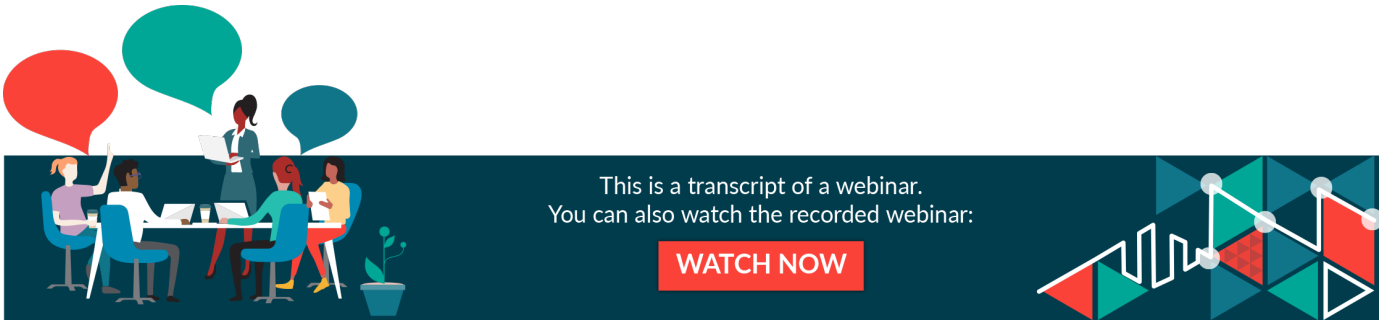

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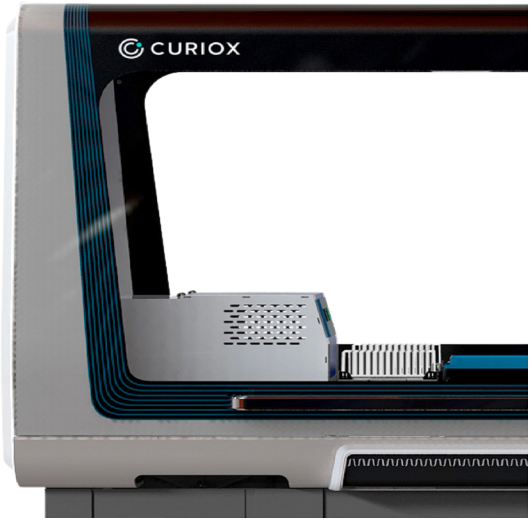

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CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Early-stage considerations for accelerating cell and gene therapy commercialization

Sabrina Carmichael

The cell and gene therapy field continues to advance at an unprecedented pace. In keeping up with the complex process development landscape and changing regulatory requirements, emerging biotechs and early-stage organizations, including academia and research institutions, face unique challenges. This article will provide the essential knowledge and practical insights needed to accelerate the development of safe and effective cell and gene therapies.

Cell & Gene Therapy Insights 2023; 9(8), 1065–1072

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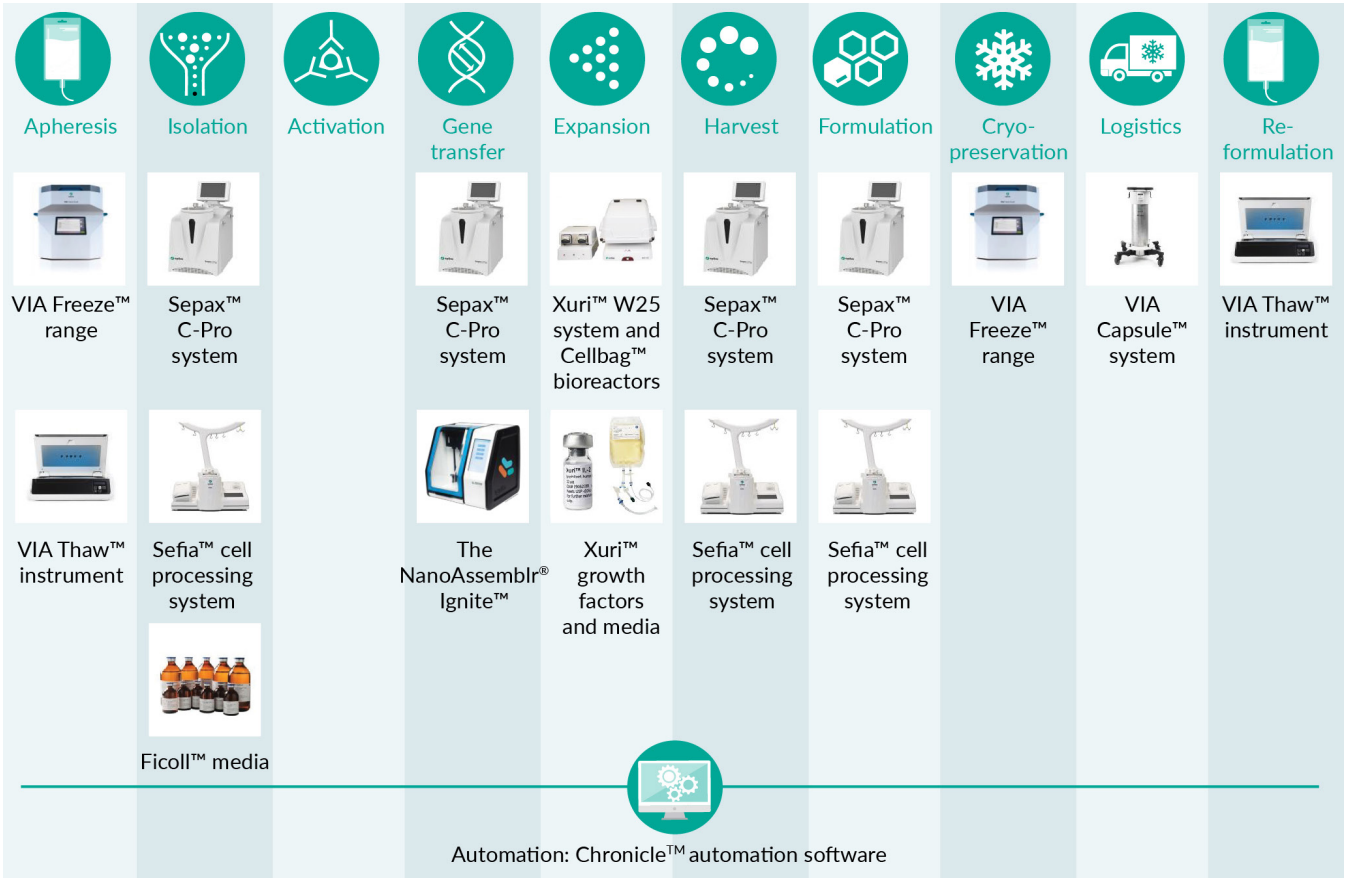
CELL THERAPY WORKFLOW

The generic cell and gene therapy workflow typically begins with a clinician consultation to discuss therapy options. Once a cell therapy is chosen, apheresis and concentration of cellular material are completed, prior to cryopreservation if needed. Once at the manufacturing facility, production of the therapy begins. This includes stages of isolation, activation, gene transfer, expansion, harvest, formulation, and cryopreservation. Then, at the clinic, thawing, reformulation, and infusion occur.

This is a complex process requiring specialized equipment, which likely should be closed and automated to allow for a repeatable and robust process. Cytiva offers a full cell therapy solution, with a complete range of equipment for different parts of the workflow (Figure 1). Chronicle software links each of these pieces of equipment together, along with third-party equipment for remote monitoring, electronic batch records, and inventory.

FIGURE 1

Cytiva's cell therapy solution.



ENTERPRISE SOLUTIONS

The Enterprise Solutions Group can help you understand an end-to-end workflow and how to scale up or scale out, or prepare for clinical trials or commercialization. They enable functionally closed, automated, and compliant cell therapy manufacturing by the use of flexible equipment that can support multiple cell therapies. This group can help propel you to your next milestone, whether that be series funding, acquisition, commercialization, or initial public offerings (IPOs), by using turnkey and customizable offerings.

There are three pillars within Enterprise Solutions: the FlexFactory™ platform, Fast Trak™ services, and KUBio™ Facility. The FlexFactory platform includes instrumentation, a dedicated project team, and training. The dedicated project team looks at suite designs, GMP and compliant closed and automated systems, and manages the day-to-day of your project. Fast Trak services complete process development and training. This involves closing and automating manual and open processes, media development, and process optimization. The KUBio facility is a greenfield facility with prefabricated GMP-grade manufacturing suites. This is modular, expandable, and offers box-in-box solutions.

FAST TRAK SERVICES

Fast Trak services incorporate process development, training, applications content development, and support services. On the process development side, the services are available at any scale, from early stage to late stage, including media screens, equipment comparisons, validation runs, and engineering

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runs. The services utilize the Cytiva portfolio, alongside some third-party instrumentation. Standard operating procedures (SOPs) will be developed along with the process, and technology transfer can be performed either at a Cytiva facility or a customer facility.

On the training side, standard courses are available both in-person and online, based on both theory and practice, in addition to customizable options. In-person training to operate pertinent instrumentation for manufacturing a cell therapy with live cells is also offered. All FlexFactory training is covered by Fast Trak.

Another service is applications content development, which is an internally resourced project around market-applicable data generation. This can cover a large range of applications. In terms of support services, Cytiva's clinical partnership team typically works with early-stage customers to advance their pipeline. There are also scientific and regulatory consultation services along with process design.

CELL THERAPY & VIRAL VECTOR PROCESS DEVELOPMENT SERVICES

Cytiva makes innovative solutions to support customers throughout their therapy journey, from the research stage through to market applications, with different services to help at each stage and phase.

Various capabilities for cell therapy process development are available, from small-scale projects, to full technology transfer and validation runs. Typically, Cytiva work with chimeric antigen receptor (CAR) T/T cells, tumor-infiltrating lymphocytes (TILs), natural killer (NK) cells, embryonic stem cells, induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), and dendritic cells, though this is not an exhaustive list.

Upstream capabilities include design of experiments, process development, cell culture media screening and development, technology transfer, and scale-up/scale-out. Downstream capabilities include cryopreservation, final formulation, and formulate/fill improvements. Analytical capabilities are also offered, including the development of SOPs, assays, and analytical transfer. Similar process development capabilities are also available for viral vectors, across upstream, downstream, and analytics, for lentiviral vectors and adeno-associated viral (AAV) vectors.

FAST TRAK SERVICES CASE STUDY

This case study provides an example of internal applications content development around TILs. When developing this method, the typical cell therapy workflow was observed, pulling in select pieces of Cytiva equipment for each step, as demonstrated in Figure 2. This end-to-end solution is functionally closed and allows ease of use with automation throughout.

Results of the expansion step of TIL cells are shown in Figure 3. Data is from the Sefia S-200 cell processing instrument. For TIL culture, tumors were resected from a tumor site at 0.25–0.5 g and collected in sterile conditions. Collected tumor tissues were shipped to Cytiva, and the tumor tissue was disaggregated and the cells were isolated in a closed, temperature-controlled, automated device. The extracted cells were cultured in Xuri EM media with 6000 IU/mL Xuri IL-2 for 7–21 days to achieve 1e7 TILs. Primary TILS were further expanded using a rapid expansion protocol (REP) by stimulation with anti-CD3 antibody and irradiated peripheral blood mononuclear cells (PBMCs) in a GRex 100 M CS. REP TILs were then transferred to Xuri bioreactor to further expand to ~1E10 viable TILs. The isolated cells pre-REP show high viability, and cells were successfully expanded.

The results from this case study, shown in Figure 4, demonstrate that REP TILs were successfully harvested, washed, and formulated using the Sefia S-200 cell processing instrument from Cytiva.

FIGURE 2

Cytiva equipment used in TIL process development.

VIA Thaw™ L100
- Dry automated thawer
- Used for point of care
- Used for cell thawing between pre-REP and REP

Sepax™ C-Pro
- Closed and automated cell washing system
- Used for washing cellular product upstream or downstream

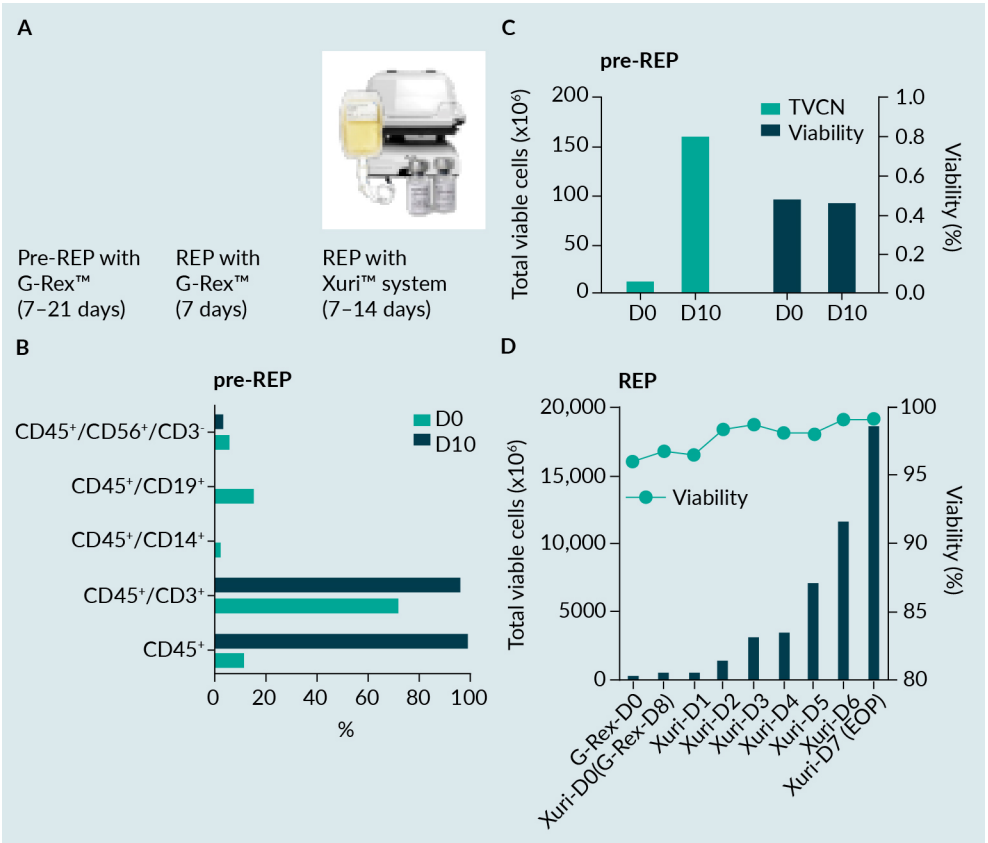
Xuri™ W25 cell expansion system
- Closed and automated cell expansion system
- Scalable from 300 ml to 25 L in functionally closed system
- Automated methods allow for ease of use

Sefia™ S-2000
- Closed and automated cell washing system
- Used for washing cellular product upstream ot downstreram
- Can handle volumes up to 10 L for harvesting and final formulation

VIA Freeze™ Quad
- LN2 free controlled rate freezer
- Used for cryopreservation of TIL products

FIGURE 3

***In vitro* expansion of the representative TILs isolated from tumors.**



(A) Outline of the cell culture workflow. **(B)** Pre-REP cell expansion. **(C)** Subpopulation of the cells during pre-REP. **(D)** REP TIL expansion.

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FIGURE 4

TIL harvest and formulation.

A

B

Sample	Viability (%)	Viable cell density (1e6/mL)	Volume (mL)	Total viable cell number	Recovery rate (%)
Prior wash and formulation	98.53	2.51E+06	5,000	1.25E+10	89.52
Post wash and formulation	94.10	7.48E+07	150	1.12E+10	

(A) Final TILs product post-Sefia processing. **(B)** High recovery rate and viability post-Sefia processing.

Fast Trak training courses are available in-person and online. The advanced cell therapy technology course (CELLT1) is a 3-day course run around the world, or as an online version (eCELLT1) covering cell manufacturing. The UNI2 online course covers UNICORN software and method editing for cell therapy. Customizable courses are also available to be amenable to specific company needs, and are ideal for new hire training. Fast Trak centers are located globally, in Marlborough, Toronto, Switzerland, and Shanghai.

SUMMARY

To accelerate the development of safe and effective cell and gene therapies, critical considerations in process development must be explored, and strategies for scale-up and quality control must be thoroughly outlined. To successfully translate to commercial-scale manufacturing, commercialization should be considered from the very beginning of cell and gene therapy development, to ensure commercial readiness even at the early stages.

Q&A

Sabrina Carmichael

Q How would you maintain closed operations within 3–4 pieces of equipment for CAR T cells from isolation to formulation or harvest? Prodigy seems to operate from isolation to harvest in one piece of equipment—I assume as a closed operation?

SC: Your assumption is correct—that is a closed system.

With a modular approach, we use many of the tools that are available, such as sterile welding. All of the kits that are on our systems, whether it be the Sepax, the Sefia, or the Xuri bags, have PVC tubing that allows them to be sterile welded. This is also useful if you need to scale up. If you are in Xuri, and want to go from using a 2 L bag to a 10 L bag, you can sterile weld that 2 L bag using the PVC connection right onto a 10 L bag for your next culture. Tools are available that work with the more modular approaches.

Q I am in the early stage of development and my process. Can Cytiva help me to develop my process from there?

SC: Yes, we work all the way through the early stage to late stage. We want you to understand the thought process about going from early to late stage. We are happy to work with you in the early stage, and we have the know-how and the knowledge to tell you where you need to go to.

Even when developing your early-stage process, we will talk about what will happen when it's time to scale up and help you think about downstream processing at a larger scale in the future. We are happy to work with you throughout all of those stages.

Q What type of process development does Fast Trak typically work on?

SC: We typically work on things in the cell therapy space, including CAR T, TILs, and NK cells. We look at manufacturing workflows, but we also have other capabilities, such as media screens or equipment comparisons. We are happy to look at any type of process development that involves cell therapy and different applications, whether it be this massive undertaking of a project that takes 9–12 months, or simply optimizing a single parameter on one of our instruments.

Q Can you use other bioreactors than the Xuri?

SC: Yes—there are many bioreactors available on the market. At Cytiva, we are focused on the Xuri, but within Fast Trak services, we do like to cater to the customer. If there is something that you want us to use, we are happy to do so, whether we already have it in the lab or can procure it. We are always looking to use different equipment available to complete the best process for that customer.

Q To what capacity can you scale up a process?

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SC: The current scale capacity for the Xuri is <25 L. Within the cell and gene therapy portfolio, there are other pieces of equipment within Cytiva that go up to 50 L. On the bioprocess side, some operate at 2,000 L. This might be a little bit aggressive for cell therapy at the moment, but we are always open to doing new things.

BIOGRAPHY

SABRINA CARMICHAEL is the Fast Trak Global Technical Leader at Cytiva. Fast Trak focuses on process development, training, and applications content development. In her role, Sabrina creates content for training courses and delivers those courses to customers interested in cell therapy manufacturing. She also engages with customers for process development, working with them to optimize and automate their cell therapy manufacturing processes. Prior to her work at Cytiva, Sabrina was a Lab Manager and Lead Research Technologist at the Center for Platelet Research Studies at Boston Children's Hospital. At Boston Children's she worked on clinical trials related to pediatric and adult patients with various platelet disorders, focusing on flow cytometric analysis. Sabrina has also worked for The State of Massachusetts Department of Public Health as a Bacteriologist in an HIV and Hepatitis Diagnostics lab, as well as a Molecular Technologist for Genzyme Genetics focusing on prenatal diagnostics. Sabrina has a background in biology and biotechnology with an MSc in Biotechnology from Johns Hopkins University.

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AUTHORSHIP & CONFLICT OF INTEREST

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CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

How reducing labor time can save on your cell therapy manufacturing costs

Yen Kong, Jeeheon Kang & Janôt Schoep

In the early clinical development stage of autologous cell therapy manufacture, labor costs often dominate the overall cost of goods sold. However, as manufacturing scales out, the cost of materials—including media and reagents, consumables, and viral vectors—increases as a percentage of the total cost of goods. Economies of scale can be achieved with autologous cell therapy processing—but in order to widen access to these life-saving treatments, it's crucial that manufacturers utilize solutions that can support reductions in labor costs. In this article, three experts explain how to reduce labor time for certain critical steps of autologous cell therapy manufacturing by using automated and closed systems.

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ASSESSING CELL THERAPY MANUFACTURING COSTS

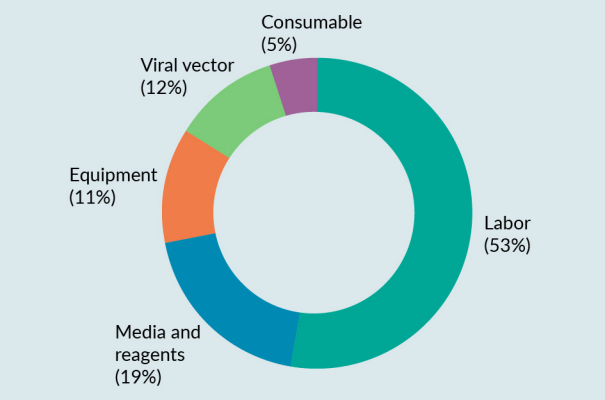
It may come as a surprise that when considering the overall costs associated with a generic autologous CAR-T therapy manufacturing process, the costs of labour dominate at 53% (Figure 1). Our own analysis suggests that by eliminating manual processing steps, labor time can be reduced and thus lower manufacturing costs. A recent analysis published by a team from the Boston Consulting Group came to a similar conclusion, and further noted that labor costs have to be reduced by 60% in order for cell therapy to become cost-effective [1].

But how can the industry achieve this 60% reduction? One approach to help reach this goal lies in replacing manual processing steps, reducing human touch points, and digitizing manufacturing and quality control documentation. To this end, Cytiva has developed solutions covering the vein-to-vein chimeric antigen receptor (CAR-T) cell workflow. This includes closed and automated systems that can replace manual processing steps, and also Chronicle™, a cloud-based software system that digitizes all manufacturing documentation.

This article will focus on how automated systems can address challenges in specific unit operations, covering cell processing

FIGURE 1

Breakdown of a generic autologous CAR-T therapy manufacturing process and with a target throughput of 10 therapeutic doses per year.



systems for cell isolation, cell expansion, and cryopreservation.

AUTOMATING MONONUCLEAR CELL ENRICHMENT

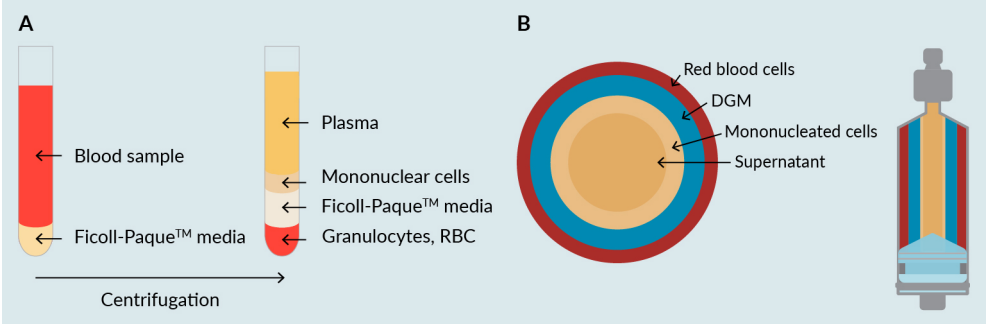
Cytiva's two cell processing units, the Sepax C-Pro and Sefia systems, can reduce processing time by automating the enrichment of mononuclear cells. In a typical process of mononuclear cell enrichment, the density gradient medium (DGM)—also referred to as Ficoll—is used to separate mononuclear cells from granulocytes and red blood cells (RBCs) by density. Ficoll is available in research use only (RUO) or GMP grades, and with different densities, but it is standard protocol to use Ficoll with a density of 1.077 g/mL. In a manual process, Ficoll is first pipetted into a conical tube, and the blood sample is then gradually and carefully overlaid onto the Ficoll to minimize mixing.

The conical tube is then centrifuged with a swinging bucket centrifuge, separating the blood sample into several layers (Figure 2A). On both the Sepax C-Pro and Sefia systems, the Ficoll isolation process is closed, and the fluid handling, blood overlaying, buffy coat extraction, and washing steps are all automated. A key technology used is a spinning separation chamber, which generates centrifugal forces to form vertical columns of the different fractions (Figure 2B).

The Sepax C-Pro system uses the NeatCell C-Pro protocol and a CT-90.1 single-use disposable kit. However, platelets can reduce the recovery of cells with Ficoll isolation, so we recommend running the PlateletFree C-Pro protocol with a CT-60.1 disposable kit to first deplete the platelets from the blood product before running NeatCell C-Pro. With these two procedures, we are able to process up to 880 mLs of initial product leading to a final volume of between 8–20 mLs, or another option of 45 mLs.

FIGURE 2

A) manual isolation of target cells from apheresis B) automated centrifugation and fluid management.



DGM: Density gradient medium; RBC: Red blood cells.

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On the Sefia, the two procedures found on the Sepax C-Pro are combined into one named PremierCell, which uses a different CT-300.1 single-use disposable kit. The Sefia kits that are used with the PremierCell protocol involve a larger kit containing more bags and tubing as it combines two protocols into one. With the PremierCell protocol, there is also an additional dilution step included in the process, which allows processing of thawed frozen apheresis products.

The handoff between the two separate Sepax protocols is seamless and fully automated. With PremierCell, the range of final volume is from 20–200 mLs with the option of splitting the final volume into two bags. With manual processing, the time taken to perform these steps is around 4 h and 20 min in total. When closing and automating the process with either the Sepax C-Pro or the Sefia, the total time can be reduced to 2.5 h, which translates to a 43% reduction in processing time (Figure 3).

REDUCING TIME DURING CELL EXPANSION

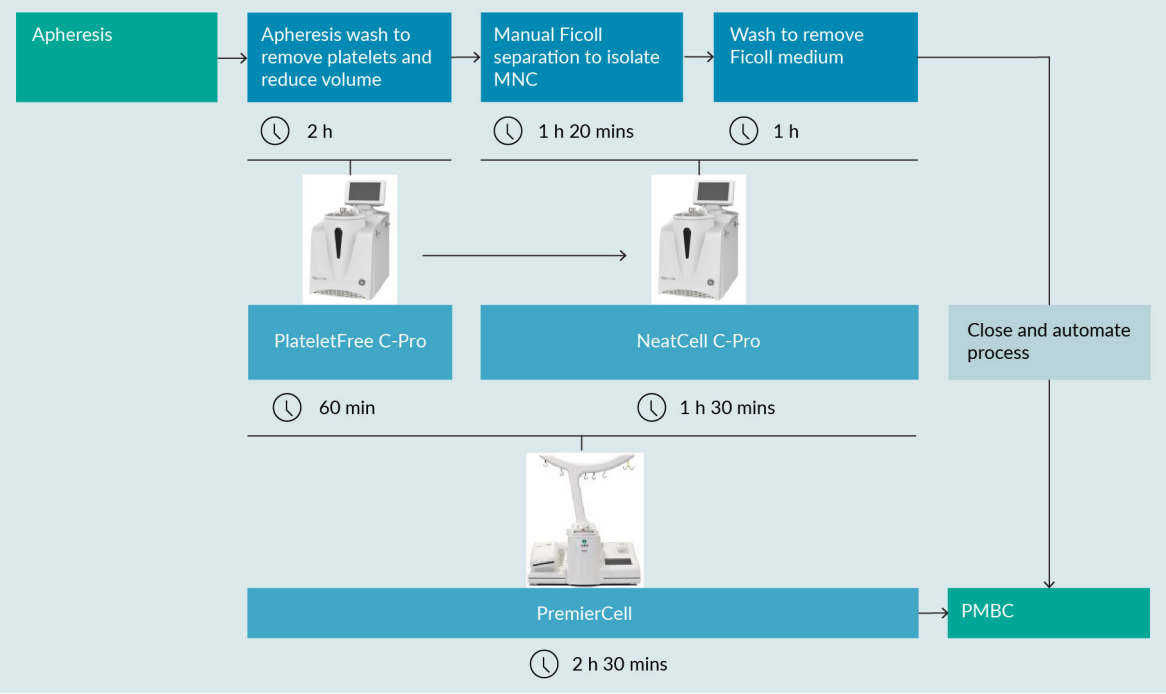
During the cell expansion step, the aim is to secure a number of cells higher than the dose required for the treatment, and it is necessary to achieve consistent results that satisfy all relevant regulatory requirements. The expansion step must also be functionally closed to protect from contamination due to mishandling—with the current trend being to use a single-use container to prevent cross-contamination.

Expansion should be scalable, make it easy to reach the target dose, and be automated to prevent human error in the current culture. The Xuri™ cell expansion system from Cytiva meets all of these requirements (Figure 4). The single-use Xuri Cellbag is used as a single-cell container to keep the process closed, and it is possible to culture from small amounts of 0.5 L to a maximum of 25 L.

Through the associated UNICORN software, it is possible to manage the protocol

FIGURE 3

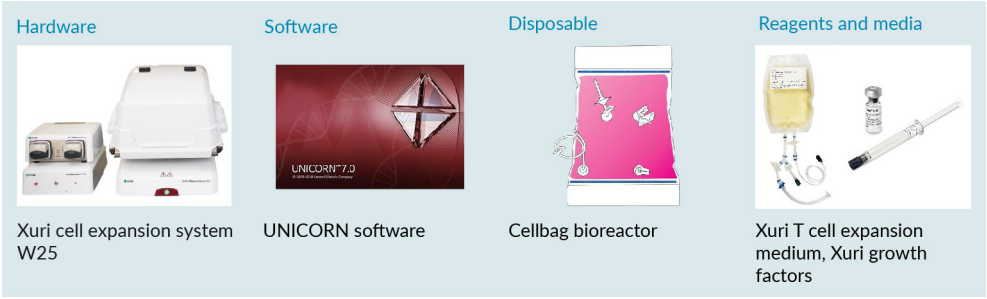
Total time reduction achieved by automated versus manual cell enrichment.



MNC: Mononuclear cells; PMBC: Peripheral blood mononuclear cell.

FIGURE 4

Configuration of the Xuri cell expansion system W25.



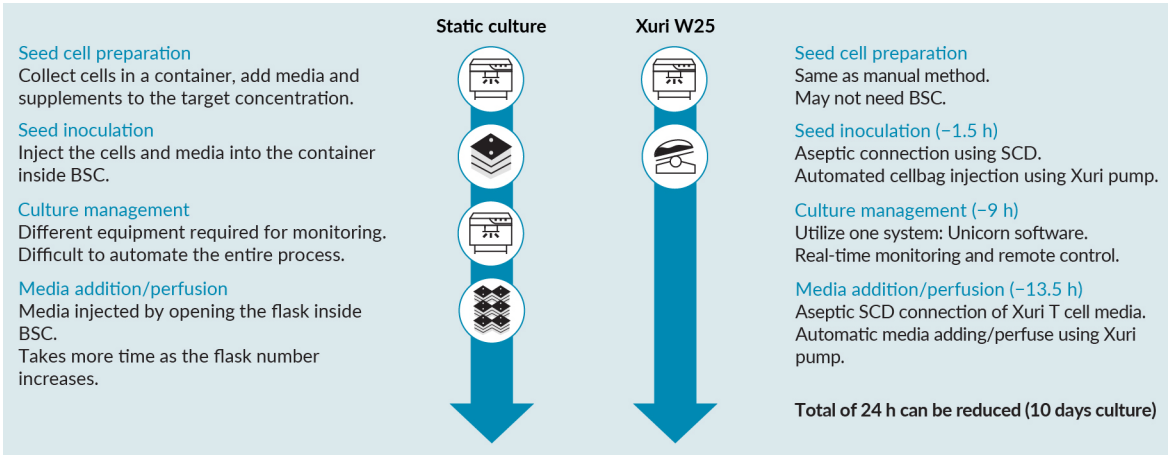
and control the operation, and all the qualification and validation guides are provided. The Xuri hardware system and UNICORN software enable a higher level of automation. Cells are able to grow safely inside the single-used Xuri Cellbag, which is functionally closed. In particular, the perfusion filter built into the Xuri Cellbag enables optimized perfusion culture without additional equipment, enabling the best culture conditions. The Xuri T cell expansion media and growth factory reagents can be connected directly and aseptically to the Xuri Cellbag, simplifying the culture process.

Figure 5 demonstrates how the Xuri expansion system can effectively reduce labor time as compared to manual static culture based on 10 days of culture.

Looking first at manual static cultivation, cells are prepared in a specific concentration, and the process of containing them with media and supplement is done inside a biological safety cabinet (BSC). The process of inoculation is also performed inside the BSC. For the process of managing the culture after inoculation, the cells will be shuttled between a CO_2 incubator and BSC. The culture markers are monitored through sampling and other monitoring equipment. As the scales grow larger, the number of culture containers increases, and the labor time will increase exponentially.

FIGURE 5

Static culture versus the Xuri W25.



BSC: Biological safety cabinet ; SCD: Sterile connection device.

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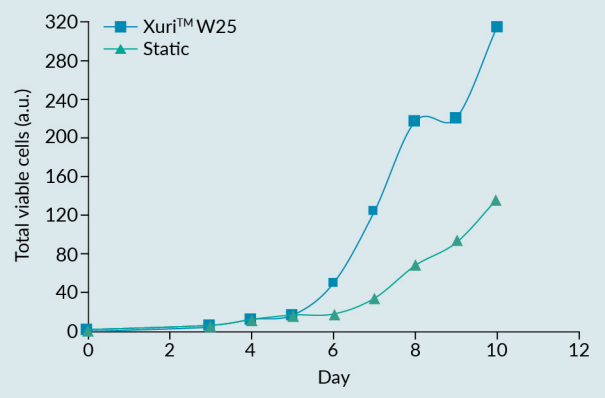
With the Xuri system, the seed preparation process can be carried out in a BSC in the same way as the manual process. The cells are seeded into the single-use Xuri Cellbag using a sterile connection device (SCD) and can be automatically injected using the Xuri pump. Compared to using a manual method in a BSC, up to 1.5 h can be saved.

The Xuri Cellbag automatically measures and recovers its culture markers such as pH, dissolved oxygen, temperature, and weight in real time through the UNICORN software until the end of culture. The Xuri Cellbag can be directly connected to the Xuri Cellbag media bag with an SCD, and adding media is quick and convenient using the Xuri pump. Since there is a perfusion filter built into the Xuri Cellbag, perfusion culture is possible without any help of additional equipment, so the cells can grow even higher than their original density.

Comparing the two methods of culture overall, a total of 24 h of labor time can be saved on 10 days of culture. Compared to the manual static culture, Xuri culture also shows superior growth kinetics thanks to the perfusion culture method (Figure 6). In the Xuri expansion systems cells grow from start to finish in a single-use closed vessel, whereas static culture required a total of 53 tissue culture flasks.

FIGURE 6

Cells cultured on Xuri systems showed superior growth kinetics compared to manual static culture.



AUTOMATING YOUR CRYOPRESERVATION PROCESS

Cryopreserving cells within your workflow provides a number of benefits, including:

- Extended shelf life—delivers long term cell viability and function; minimizes genetic changes;
- Logistical benefits—timeframe for cell shipment is prolonged;
- Treatment consistency—patient can receive multiple treatments with the same batch of cells;
- Improved COGs—scalable manufacturing process;
- Quality control—allows release testing of the manufactured product;
- Supply stability—greater flexibility in therapy timing.

The VIA Freeze™ instrument from Cytiva provides a number of benefits for cryopreservation. Customizable freeze profiles combined with precise temperature control maintain optimal cell function and viability. The user can customize their freezing profile depending on the cell type or cryocontainer in use in order to maintain consistent quality. Conduction cooling removes heat evenly so that every sample experiences exactly the same cooling rate. Finally, the liquid nitrogen-free cryopreservation approach enables use in cleanrooms.

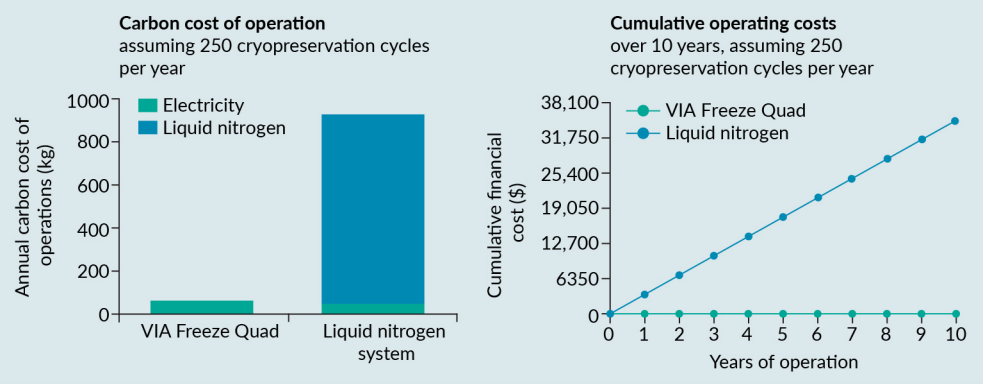
VIA Freeze also avoids the handling risks that come with liquid nitrogen, and results in no oxygen depletion or air contamination. Stirling cryocoolers cool the samples in a completely sealed heat transfer system. Additional cleanroom-compatible features include an easy to clean design.

Figure 7 shows a comparison between VIA Freeze and a liquid nitrogen system in terms of the carbon cost of operation, and also cumulative operational costs.

Turning to the question of cell recovery, cells frozen using VIA Freeze have comparable

FIGURE 7

VIA Freeze instruments reduce emissions and operating costs when compared to liquid nitrogen systems.



post-thaw recovery and viability to those frozen in a liquid nitrogen-based freezer. A study was performed, as illustrated in Figure 8, using processed cord blood bags. The experiment showed very comparable results achieved with VIA Freeze Uno—and it is important to note that in addition, VIA Freeze avoids the various drawbacks of handling, including contamination risks, and drastically reduces cost.

Finally, Cytiva's Chronicle automation software combines production, quality, logistics, inventory management, and maintenance inside of one system, streamlining processes and ensuring everything is captured in a single system (Figure 9).

CONCLUSION

Labor costs alone comprise more than half of the total cost of autologous CAR-T therapy manufacturing processes, and solutions to reduce this cost burden are critical for the industry.

When comparing operator tasks in a standard process versus a process using Cytiva systems for specific steps, utilizing automated and closed systems resulted in reduced time and costs without compromising quality and consistency—while also avoiding drawbacks such as increased contamination risk from manual handling steps.

FIGURE 8

Post-thaw recovery and viability were comparable between the standard LN2-based method and the VIA Freeze.

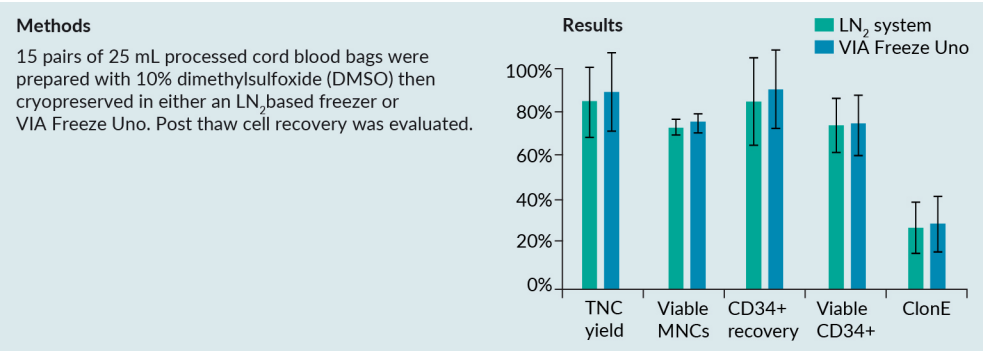


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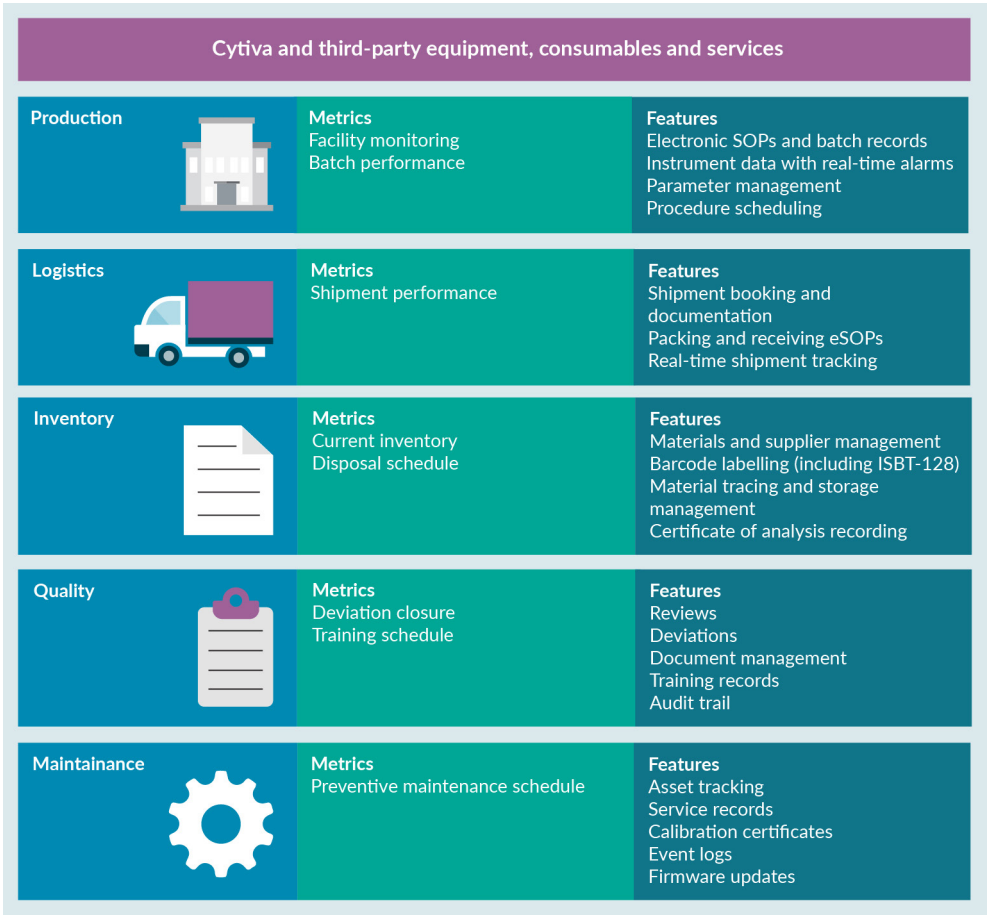
FIGURE 9

Chronicle software at a glance.



ASK THE EXPERTS



Elisa Manzotti, CEO & Founder, BioInsights, speaks to **Yen Kong,** Field Application Scientist for Cell Therapy, USCAN, Cytiva, **Jeeheon Kang,** GM Cell Therapy FAS Leader, APAC, Cytiva and **Janôt Schoep,** Field Application Specialist for Cell Therapy, EMEA, Cytiva

Q Are there any other differences between PremierCell and the two procedures, PlateletFree C-Pro and NeatCell C-Pro, or is it just a combination of the two?

YK: It is indeed a combination of the two procedures, but there are additional features baked into PremierCell. One is a dilution step which allows frozen units to be processed. Two, the temperature at which the process is run can be controlled, and this can further improve recovery by stabilizing the Ficoll density. Three, the range of adjustable process parameters has been expanded to allow further flexibility and tuning of the process to your application. Finally, there's an option to split your final product into two doses.

Q What are the benefits of perfusion culture versus fed-batch?

JK: In a fed-batch culture method, media can be only added and not reduced. In the cells inside the fed-batch culture, metabolites like lactate and ammonia are accumulating. Using the perfusion culture method, metabolites such as ammonia and lactate are reduced to keep the cells healthy and allow higher growth.

Q Can the VIA Freeze only work with cryovials?

JS: The VIA Freeze instruments can work with cryovials. We can also freeze down cryobags, for example, or even 96-well plates and straws, if considering other areas like the fertility industry.

Q You mentioned that your cell isolation systems can process up to 880 mL of input material. How many cells can be isolated with your devices?

YK: Although our cell processing systems can process up to 880 mL of initial material, our Ficoll volume is fixed, so a maximum of around 12 million total nucleated cells can be processed.

Q Which cell types are suitable for the Xuri Expansion System?

JK: Xuri can be applied to most of the immune cells, such as T cells, NK cells, and even induced pluripotent stem cells or mesenchymal stem cells. It can also be applied to HEK cells, as it's already used in the bioprocess world. It's very useful for cells to have that gentle rocking, and with the perfusion methods combined, it's a very powerful tool for cell and gene therapy.

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Q What is the minimum target temperature that can be set up on the VIA Freeze?

JS: The VIA Freeze instruments can all reach a minimum temperature of −100°C.

BIOGRAPHIES

YEN KONG is currently a Field Application Scientist based in Marlborough, Massachusetts supporting cell therapy customers in the US and Canada on their process development needs. Before his current role, Yen was a Development Scientist in R&D involved with developing the next generation bioreactor for closed and automated expansion of cell therapies.

JEEHEON KANG received a PhD in Genetic Engineering from Seoul National University, worked as a project manager at Asan medical center, and has been working as a Cytiva Cell Therapy Field Application Specialist for four years. Based on her knowledge of the cell therapy equipment from Cytiva, she is conducting customer seminars, trainings, and demonstration experiments to support customers for better therapy.

JANÔT SCHOEP is currently working at Cytiva as a Field Application Specialist. Before working at Cytiva, Janôt Schoep was working inside a GMP manufacturing facility, being hands-on with patient material to create several cell therapies that are currently in clinical and commercial phase. He has experienced the benefits of using automated instruments that can standardize and close the manufacturing process and reduce contamination risk.

AFFILIATIONS

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REFERENCE

1. Khang M, Suryaprakash S, Kotrappa M, *et al.* Manufacturing innovation to drive down cell therapy costs. *Trends Biotechnol.* 2023; S0167-7799(23)00127-0.

AUTHORSHIP & CONFLICT OF INTEREST

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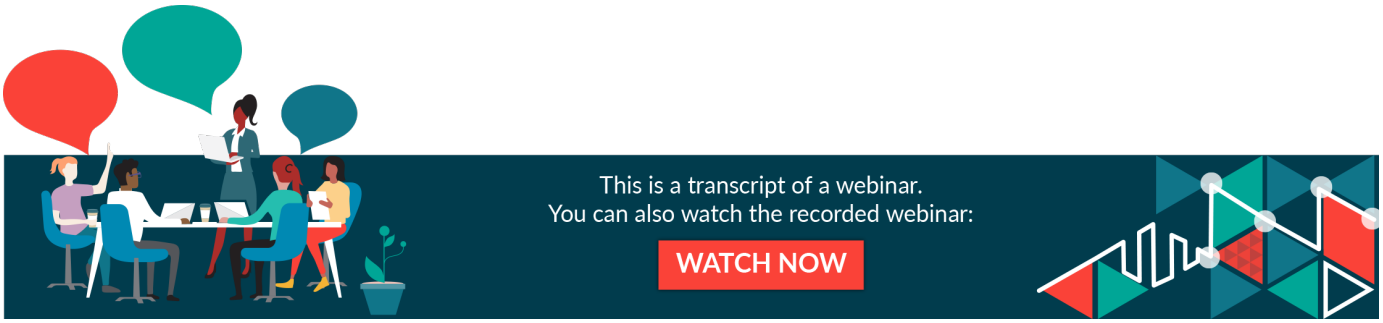

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Pall + Cytiva

Turns out, we have chemistry

Sometimes, two powerful forces are even stronger together. That's what we discovered with Pall Life Sciences and Cytiva: united as one company, we now deliver the broad biopharma solutions and expertise our customers need in their journey – from discovery to delivery. This is chemistry where it matters most.

cytiva.com/perfectmatch

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

CY37880-30Jun23-AD

CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

High-throughput process & analytical platforms to accelerate gene therapy scale-up

Patrick Starremans, Kenneth Warrington & Nithya Jesuraj

For revolutionary gene therapies to reach their full therapeutic potential, high-throughput process development workflows are needed to keep pace with the ever-changing landscape of capsids and payloads. In addition, many of the current analytical testing methods are either unable to match the pace of process development, use too much material, or are inherently unsuited to support various unit operations. The development of robust fit-for-purpose analytical platforms must match the pace of innovation the industry is seeing. While we continue to discover the broad applications of the various AAV serotypes, their distinct biological properties require tailored, stage-appropriate analytical methods which are able to provide higher resolution using less material, and provide novel insights to keep pace with the ever-increasing speed of process development activities.

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Resilience was founded in 2020 as a technology-focused company dedicated to broadening access to complex medicines. By continuously advancing the science of biopharmaceutical manufacturing development, Resilience was formed to address two defining challenges. These include the need for manufacturing supply chains that can withstand disruptive shocks and safeguard health security. In addition, biopharmaceutical manufacturing has not kept pace with scientific advancement, preventing new discoveries from reaching patients at speed or scale. To address these challenges, Resilience focuses on five different modalities: cell therapy, gene therapy, nucleic acids, vaccines, and biologics.

Lacerta Therapeutics was founded in 2017 to develop novel AAV gene therapies for central nervous system (CNS) applications, as well as to innovate in specific areas, including rational and combinatorial capsid engineering platforms. Lacerta developed the OneBac

AAV manufacturing platform based on the insect cell system with the primary goal of extending the baculovirus insect system to all serotypes while striving to eliminate the need for multiple baculoviruses and large-scale plasma manufacturing.

THE DEVELOPMENT OF HIGHLY FLEXIBLE MODULAR MANUFACTURING PLATFORMS

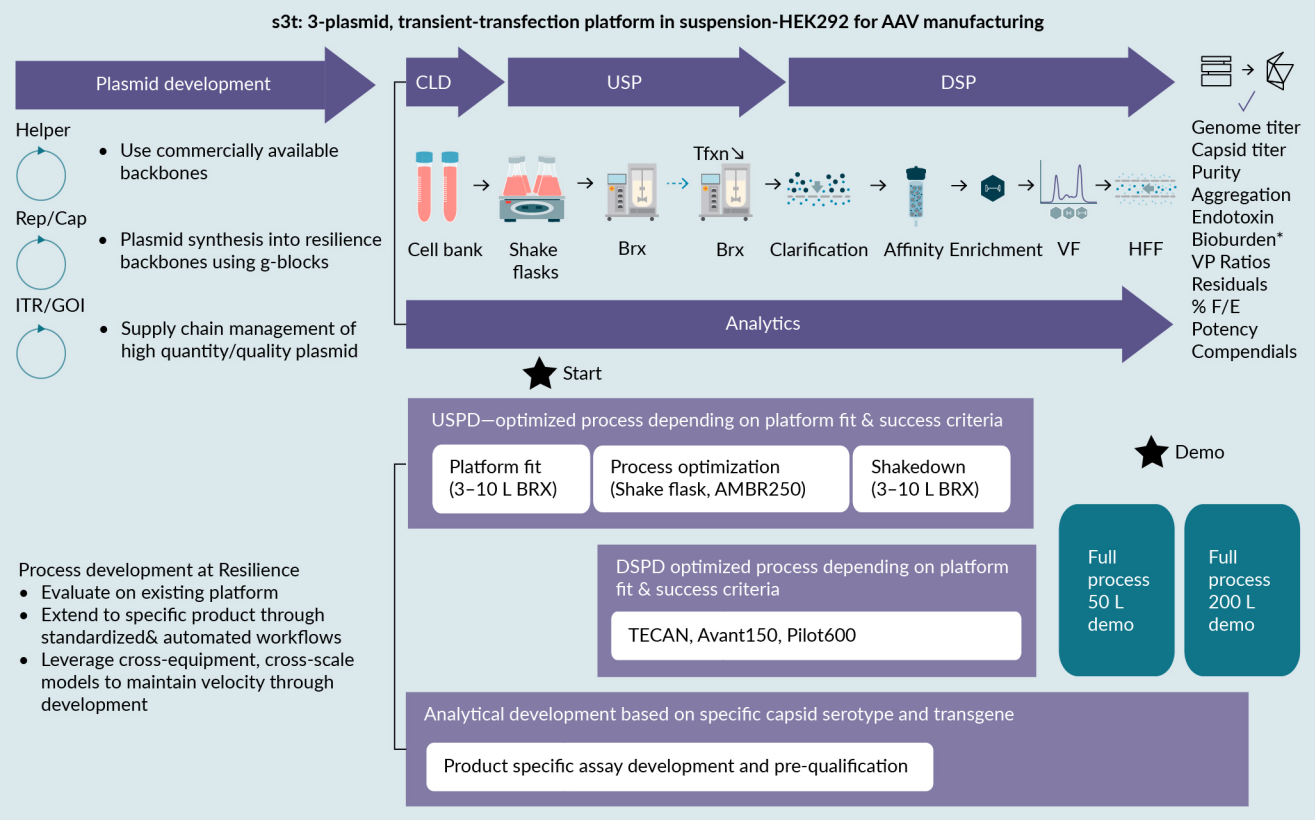
Resilience is actively investing in the development of highly flexible modular manufacturing platforms. A platform is defined as a collection of processes and protocols aimed toward accomplishing a range of operations. This is achieved by pulling from an expanding library of discrete technology modules. By combining a number of predefined modules that cover the various parts of a manufacturing process from upstream to downstream with supporting analytics, generic platforms can be generated to complete the majority of development work. The approach still needs to be fine-tuned to a specific technology, however this 80:20 approach increases the speed of process development (PD). An example of this platform approach, the s3T platform, is shown in Figure 1.

ADVANCED ANALYTICS FOR GENE THERAPY

To support these modular platforms, an accompanying analytical strategy is required. PD can only be successful alongside the right analytical tools and technologies to both guide the process and confirm the

FIGURE 1

s3T platform: a production platform with elastic unit operations or modules that allow for rapid conversion from a generic platform to a client-specific process up to the 200 L scale.



Brx: Bioreactor; CLD: Cell line development; DSP: Downstream processing; F/E: Full/empty; GOI: Gene of interest; HFF: Horizontal flow filtration; ITR: Inverted terminal repeats; USP: Upstream processing; VF: Viral filtration; VP: Viral proteins.

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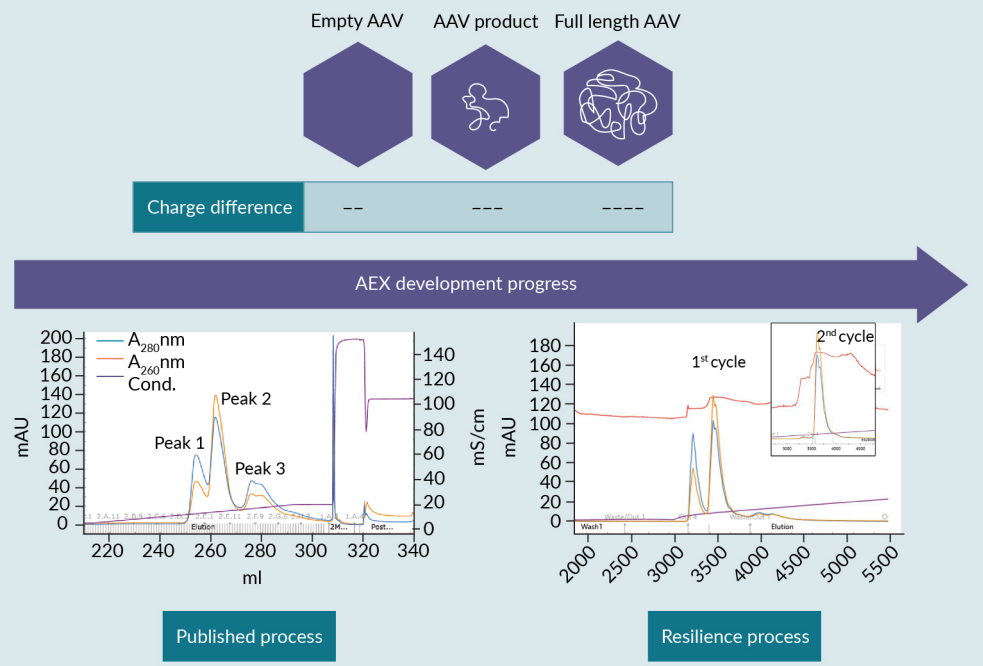
final product resulting from this end-to-end approach. Resilience is heavily invested in building an extensive analytical suite that not only covers most gold-standard technologies to interrogate AAV products, but also a significant number of orthogonal and next-generation technologies to increase knowledge and understanding of an adeno-associated virus (AAV) product. Resilience is looking at a number of next-generation technologies that replace or can be used in addition to currently outsourced time-consuming tests.

The entire analytical strategy is focused on a number of things. The first is to reduce the amount of material needed for each test. The second is to increase the turnaround and the throughput of the analytical platforms to reach the next iteration of PD faster, and the third is cost. Multi-attribute methodologies offer an increased understanding of your product with only one particular run.

Figure 2 provides an example of how to tune a particular module, in this case an anion exchange (AEX) unit operation. Significant process risk was identified during the initial tech transfer due to the AAV product being designed with a shorter transgene (2.1 kb versus 4.6 kb full length). Regular published AEX processes failed to resolve empty and full capsids due to the shorter transgene. A comprehensive exploration of AEX process levers, including conductivity, buffer matrices, elution modifiers, cycle numbers, and elution modes was completed.

FIGURE 2

AEX unit operation development.



Percentage full of DS	Published process	Optimized resolution	Bench scale (non-GLP)	Pilot scale
Mass-photometry	48%	75%	84%	85%
CDMS	N/A	N/A	93%	95%
AUC	N/A	N/A	90%	91%

AAV: Adeno-associated virus; AEX: Anion exchange; AUC: Analytical ultracentrifugation; CDMS: Charge detection mass spectrometry; DS: ; GLP: Good laboratory practice.

Full characterization of AEX elution profile was also performed to guide peak fractionation for a better purity and safety profile. The solution involved a reproducible, fully-automated peak fractionation for robust large scale GMP manufacturing. A thorough comparison of different analytical tools for empty/full quantification demonstrated great process consistency.

In AAV programs, the percent full is an important characteristic, especially with programs pursuing ever higher doses for systemic applications. Adding empty capsids adds to the risk of any adverse effects. Traditionally, the removal of empty capsids was only accomplished by using ultra-centrifugation, either via cesium chloride density gradient or by iodixanol enrichment. However, these methodologies, while effective, are challenging to translate into a GMP environment and are accompanied by significant risk. Ion exchange chromatography has recently made great strides and offers a more scalable and safer solution to reach higher percent full ratios that are needed for high dose or small volume applications.

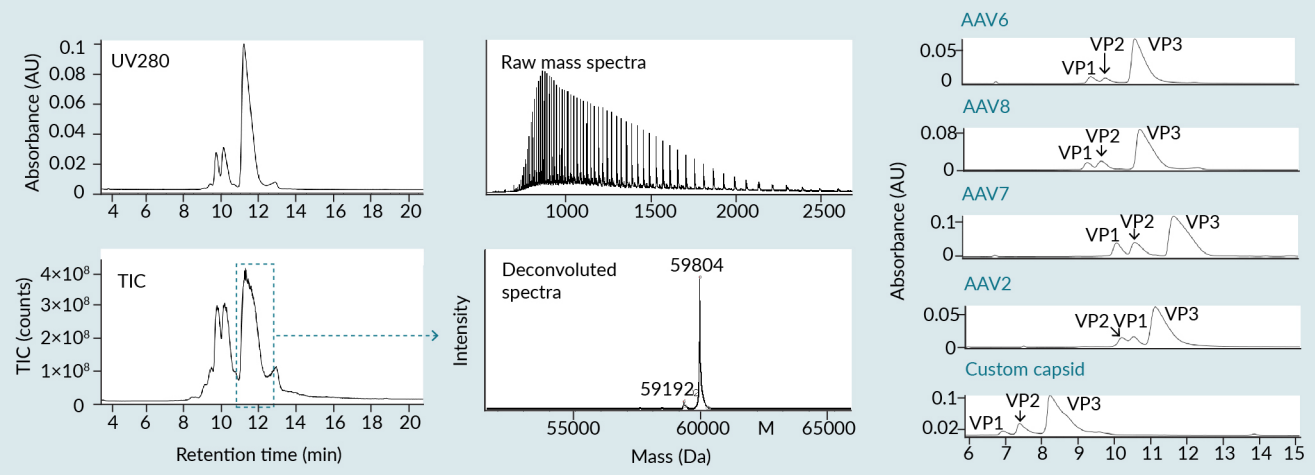
ANALYTICAL DEVELOPMENT VERSUS PROCESS DEVELOPMENT

In gene therapy programs, an eternal struggle for balance between analytical development (AD) and PD exists, as both are limited by a number of factors, including time, resolution, and sample volume. Depending on the stage of a program, a balance between fit-for-purpose and stage-appropriate activities must be found. That scope must encompass preclinical development, clinical stage, and commercial. The weight of these factors changes significantly across the process. While the balance always centers around the same parameters, improving the capability of balancing these factors in several ways is necessary, such as through platform automation.

To increase the speed of PD, interrogating multiple different attributes and variables in a single experiment can be achieved by running complex design of experiments (DoEs). Resilience uses the Ambr250 platform for rapid upstream development to interrogate a number of factors. This is coupled with an automated Tecan platform for downstream applications where, for instance, a large number of affinity elution strategies can be screened. However, this will only be successful if the analytical site can keep up by providing PD teams with data on a short turnaround. Resilience achieves this by coupling Hamilton liquid handler platforms with high-throughput digital PCR platforms. Resilience is supporting PD with high-throughput platforms, coupled with a fully automated analysis pipeline where

FIGURE 3

AAV capsid identification by liquid chromatograph-mass spectrometry.



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data is analyzed with limited interaction from an analyst and is uploaded into an electronic lab notebook (ELN), supporting greater data integrity from end to end.

CAPSID AGNOSTIC ANALYTICS

Another way to support shorter turnaround times and less challenging tech transfers is by introducing capsid agnostic analytics. In the current gene therapy environment, more programs are aiming to improve the tropism of capsids to better suit niche applications. This introduces a challenge for analytical vendors to generate capsid-specific antibodies to be used for both titer and identification. A method to exclusively confirm the serotype of the target product is required. Resilience employed AAV capsid identification by lipid chromatography-mass spectrometry to provide serotype verification and a readout on capsid protein isoforms (Figure 3). AAV capsid proteins are denatured and separated on a column with UV detection. Intact viral protein (VP)1, VP2, and VP3 capsid protein sequences are verified by intact mass. This platform technology method can be applied to most serotypes with minimal optimization and can identify mutants and other custom serotypes for which ELISAs are not available.

Another way to improve analytical strategies is by exploring multi-attribute methods (MAM). MAM allow monitoring of multiple critical quality attributes (CQAs) using on technology platform and in ideal circumstances from a single measurement. The potential benefits include lower sample needs for testing and that the single data source allows for better comparison between CQAs. Two examples of this are long read next generation sequencing and size-exclusion chromatography coupled with multi-angle light scattering (SEC-MALS).

Multiple orthogonal methods can be applied in a stage-appropriate and fit-for-purpose manner throughout the entire PD cascade. A key example is the multiple ways to look at the empty full characteristics: SEC-MALS, analytical ultracentrifugation, mass photometry, charge detection mass spectrometry, and cryo-transmission electron microscopy (Table 1).

These platform examples are designed to accelerate patients' access to therapies, and Resilience is learning from industry partners, like Lacerta, on market demands and needs in order to deliver gene therapy more quickly and safely to patients.

ONEBAC AAV MANUFACTURING PLATFORM

OneBac simplifies and improves the Sf9/baculovirus expression vectors (BEV) manufacturing process by integrating gene of interest (GOI) and helper functions, as described in Figure 4.

OneBac is universally applicable to all AAV serotypes, resulting in the preservation of capsid stoichiometry throughout the downstream purification procedure. Any AAV genome plasmid can be incorporated into the system without extensive subcloning. Minimal manipulation removes the majority

TABLE 1

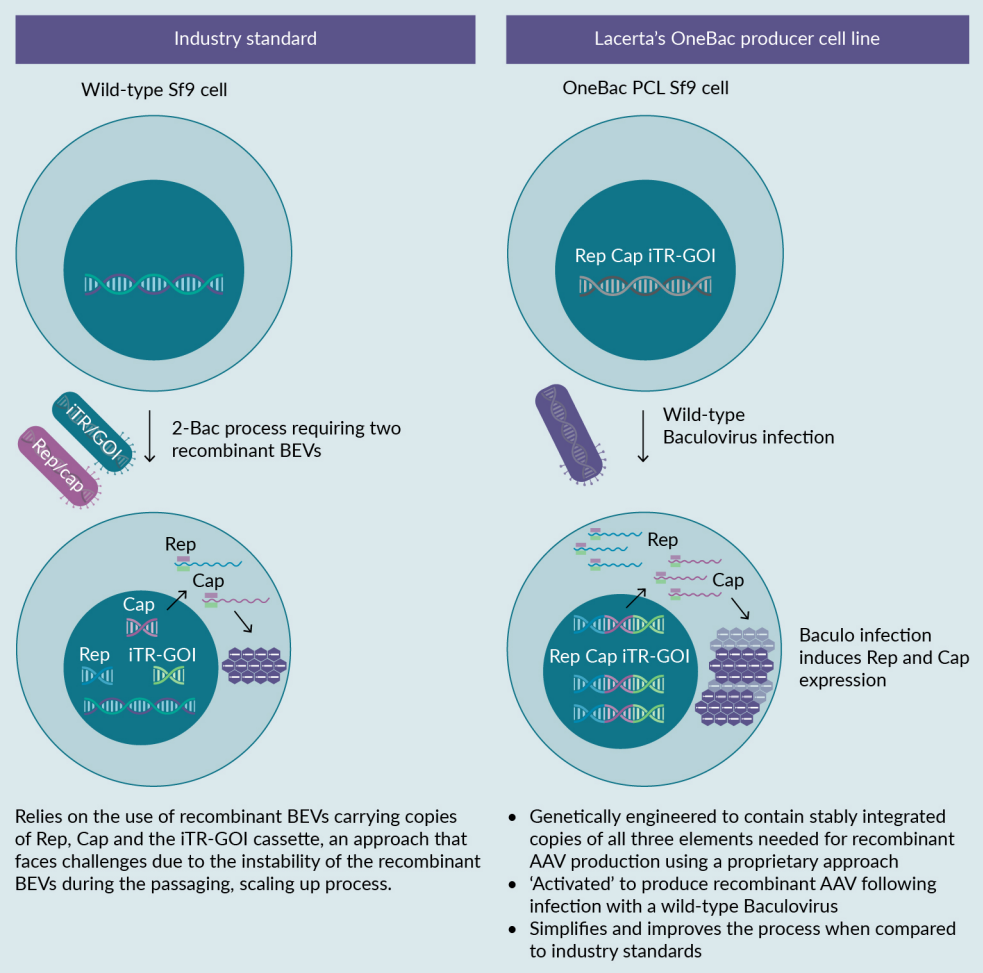
Orthogonal techniques for AAV8-hGH and AAV6-hGH material.

	% full			
	dPCR/SEC-MALS	AUC	CDMS	MP
AAV8-hGH fulls	>95	72	73	66
AAV8-hGH empties	<5	3	2	2
AAV6-hGH fulls	92	N.T.	87	70
AAV6-hGH empties	<5	N.T.	3	4

AUC: Analytical ultracentrifugation; CDMS: Charge detection mass spectrometry; hGH: Human growth hormone; MP: Mass photometry; N.T.: ; SEC-MALS: Size exclusion chromatography-multiangle light scattering.

FIGURE 4

OneBac PCL: exploiting the scalability and ease of the Sf9/BEV system to create a unique producer cell line platform.



AAV: Adeno-associated virus; BEV: Baculovirus expression vector; GOI: Gene of interest; ITR: Inverted terminal repeats.

of non-AAV genome DNA and eukaryotic sequences. The system is compatible with any recombinant baculovirus manufactured by industry-standardized methods (e.g., either transposition or homologous recombination). This eliminates the requirement for complex infection kinetics and multiple baculoviruses to achieve high yielding cultures.

CHALLENGES IN GENERATING STABLE CELL LINES

One of the challenges in generating stable cell lines is the ability to demonstrate clonality, and to rapidly increase the cell divisions required to produce a research cell bank to allow further characterization and optimization. OneBac is in a 96-well format designed to specifically incorporate small-scale screening to identify top clonal candidates. Imaging is incorporated to verify clonality and document this as the various clonal populations are expanded. This allows the identification of candidates that are selected on specific growth characteristics and AAV productivity. The system is undergoing further modifications to evaluate additional CQAs at the clone screening stage. Following the expansion in the multi-well format, the

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producer cell lines are infected with a wild type baculovirus. This allows rapid screening using standard titration methods.

One challenge of an insect cell platform is that the Sf9 cells carry rhabdovirus in certain cases, which is a heterologous infection. This method allows for the early identification of clones that no longer carry rhabdovirus and are mycoplasma free. Other challenges in working with clonal populations of Sf9 are the lack of early cell divisions, and being able to move rapidly from an adherent culture into the adaptation of a much more scalable suspension approach to the Sf9. The OneBac AAV producer system is designed with the rapid generation of stable producer cell banks in mind, with optimized cell growth to accelerate time from clonal selection to cell bank. It allows robust, reliable production over 20 passages post-research cell bank (RCB) thaw (>60 doublings).

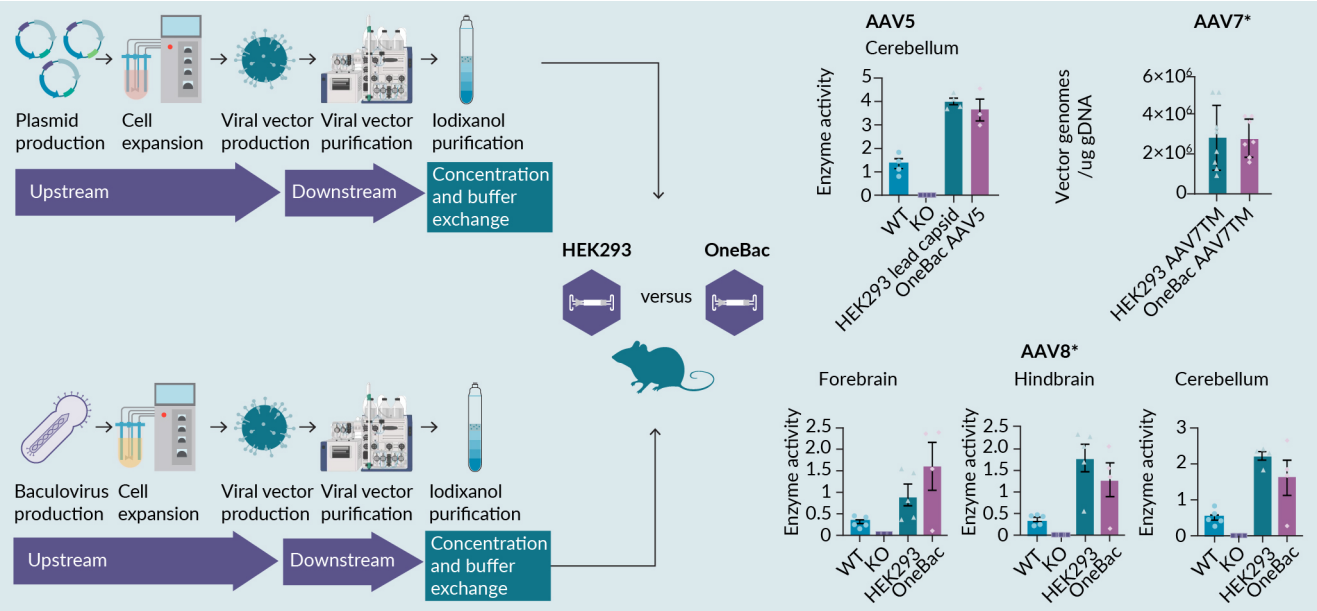
The elimination of multiple BEVs simplifies input materials and reduces variables and optimization times. A two-component system (producer cell culture and single BEV stock) simplifies basic process optimization. Primary parameters for optimization include cell growth/density at the time of infection and multiplicity of infection (MOI) of baculovirus. Both packaging and producer cell line variants retain favorable scale-up characteristics inherent to the IC/BEV system.

Figure 5 demonstrates the extensive work with transfection-based methods using the OneBac AAV producer cell line system within R&D and preclinical groups. This shows that *in vivo* within the CNS, equivalent efficacies are seen regardless of what platform is used for production.

With OneBac, Lacerta Therapeutics has been able to drive down the development time of producer cell lines for AAV and the insect cell system to 3–4 months. The BEV platform is adaptable to all serotypes and is readily scalable with minimal optimization. Consistent yields can be achieved, and enhancing the stoichiometry of downstream purification methods is possible. The technology is universally compatible with existing BEV technologies and different media types,

FIGURE 5

OneBac AAV producer cell line system: manufactured products display equivalent efficacies to HEK293-manufactured AAVs across a range of disease models.



AAV: Adeno-associated virus; KO: Knock-out; WT: Wild-type.

with familiar growth and infection kinetics. Minimal encapsulation of host cell and baculovirus DNA is seen, in addition to competitive efficacy data compared to mammalian-derived AAVs.

TRANSLATIONAL INSIGHT

The pressing need for fit-for-purpose analytical platforms for AAV manufacturing can be fulfilled by serotype agnostic analytics to reduce testing costs. Current methods to scale-up to larger bioreactors are not sustainable due to cost, transfection efficiency, and physical properties. Using an advanced platform with a reduced number of plasmids may increase vector titer by 1–2 logs, resulting in a significantly lower cost of goods (COGS). Resilience and Lacerta are working to solve the current challenges in the field and innovating to meet patient demand and reduce the overall COGS.

ASK THE AUTHORS

Kenneth Warrington, Patrick Starremans, and **Nithya Jesuraj** (pictured left to right)

Q What are the critical cell and viral substrates required for the platform?

KW: You will need a wild type Sf9 master cell bank. This will allow you to produce the various baculoviruses, whether it is bringing a gene of interest (GOI) or is the preferred wild type. You will also need the master viral bank that is produced from that cell bank, ideally a wild type so that it is applicable across multiple producer cell lines. The producer or packaging cell line will be carrying the various AAV helper functions.

Q With two AEX cycles of purification of AAV, what was the yield?

PS: We typically see around 55–65% recovery over two cycles. It varies a small amount by serotype. In some cases, we have seen higher than 65%. The loss over the second cycle is very minimal.

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Q What is the best way for upstream PD to get quick results back, such as titer capsids and so on?

PS: The best way depends on the purpose of your upstream experiment. Ideally, we focus on titer turnarounds first, which is where the high-throughput digital PCR platform helps. Once you have identified conditions that you want to explore further, we can use things like an Octet to look at capsid titers. Those are also serotype agnostic. The last option is mass photometry, which provides us with a very short turnaround method, taking around 20 min to produce a read. There are also high throughput options for that available now.

Q What other vector types can Resilience support?

NJ: Our expertise within Resilience has built up over the last three years with multiple folks coming in from all facets of industry. We focus a lot on AAV and lentiviral vectors in the gene therapy franchise. However, we do have experience in retroviral vectors, oncolytic viruses and other esoteric viruses. There is a wide variety of viruses out there and we at Resilience will be able to support that. We recommend a technical call with our tech teams to make sure that we are able to solve the problem and meet your needs.

Q Are these analytical platforms available at the manufacturing sites? How do you ensure continuity through manufacturing?

PS: Most of the gold standard methods are also available in our GMP sites. At the outset of our analytical strategies, we aim to ensure that what we develop at the process development (PD) and analytical development (AD) stage can be transferred onto the GMP floor.

Q Does the platform work with engineered capsid variants?

KW: It does work with engineered capsid variants. As part of Lacerta, we develop both rationally designed and combinatorial novel variants. We are now moving some of the novel variants derived from screening combinatorial libraries forward, which have more modification in the point mutations. To date, we have not identified either a natural or a capsid variant that we have not been able to deploy the platform with.

Q Are there any differences between purification unit operations for AAV from OneBac versus transfection platforms?

KW: We have fairly standard in-house methods in our PD group and so far it has been plug-and-play. There are some slight differences in terms of lysis conditions when

working with the insect versus transfection platform, but all operations downstream of that have been the same.

Q What serotypes work with the s3T platform?

PS: Currently, we have demonstrated the suitability of the platform for AAV2, 5, 6, 8, and 9 and we are working with 7.

Q At what scales can Resilience manufacture AAV?

NJ: Resilience can support a wide variety of scales for clients, from 50–2,000 L. This is spread across our different manufacturing sites. Depending on the program, we will choose the site (or sites) that will be able to support the necessary scales and ensure success for the client.

Q What are the approximate time savings by using this high-throughput approach?

PS: That is currently still being determined. We have implemented the platform, but as Resilience is only two years old, we are still collecting data. We need to complete one or two programs before estimating the actual time saved. But in terms of turnaround times, being able to pivot from your first PD stage to your next iteration within 2–3 days is definitely much faster than having to wait 2 weeks for your analytical data to come back.

Q Does the platform work with single-stranded and self-complementary AAV vectors?

KW: Yes, it does. Historically, it was developed around the more traditional single-stranded, but one of our lead programs in our clinical pipeline uses a self-complementary vector. We have driven many of our optimization efforts around the OneBac platform, since we have significant historical data on producing that same vector using transfection-based plasmid methods. The platform is robust regardless of whether the GOI is single-stranded or self-complementary.

Q Does Resilience have capacity right now?

NJ: Yes, Resilience has capacity right now and we welcome any new opportunities to funnel in. Additionally, at many of our GMP sites, we have expansion space.

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Depending on the demand type, we would be able to expand our manufacturing capacity significantly and build out those grey spaces to manage the demand.

Q What characterization have you done on the AAV vector genome and vectors produced in OneBac?

KW: The bulk of our focus has been around a self-complementary vector and we have focused on both Nanopore as well as PacBio Sequencing. In our experience with self-complementary vectors, we are seeing >98% genome. This means there are very low levels of either host cell or baculovirus DNA and these are taking the traditional expected forms. There has been some debate in the industry around using baculovirus when it comes to genomic integrity. However, in our hands, we are seeing exactly what we would expect in our vectors, alongside all the other benefits of the producer cell line platform.

BIOGRAPHIES

PATRICK STARREMANS is Director of Analytical Development and head of Resilience's Gene-therapy AD-group. Dr Starremans received his PhD in Medical Sciences and master's in medical biology from the Radboud University in Nijmegen, the Netherlands and an undergraduate degree in Biochemistry from Zuyd University of Applied Sciences. Following his move to the US, his career has focused on molecular, biochemical and cell-based assay development for the past 20 years, starting in discovery at Harvard Medical School followed by tenures in in-vitro pharmacology at Genzyme, Sanofi, Evotec and Mitobridge finally evolving to Gene Therapy Analytical Development when he joined Voyager Therapeutics building their Process Analytics Group and later as head of Analytical Development building out their CMC-Analytical capabilities and infrastructure and helping Voyager file their first successful IND for an internal program.

KENNETH WARRINGTON has decades of broad expertise across the cell and gene therapy product development continuum from discovery through GMP-compliant manufacturing to support IND-enabling pre-clinical and early-stage clinical programs. He has deep knowledge in advanced therapy manufacturing, including live attenuated and virus-like particle vaccines, live challenge viruses, viral vectors, and cell & gene-modified cell therapies. He served on the faculty at the University of Florida, Pediatrics-Division of Cellular & Molecular Therapy, with a research program focused on AAV vector development and production. Following his transition into industry in 2008, Dr Warrington has held the lead technical operation and business development roles for global contract testing and manufacturing organizations, including Meridian Life Science, SGS Life Science, and Wuxi Apptec. He was formerly the SVP of Operations and Business Development at Biostem Life Sciences before assuming the role of chairman of the scientific advisory board and led gene & cell therapy CMC services at GenScript ProBio. Dr Warrington is currently Chief Technology Officer at Lacerta Therapeutics. He holds a BSc in Biology & Chemistry from St Lawrence University and a PhD in Pharmacology & Experimental Therapeutics from the University of Florida.

NITHYA JESURAJ is a member of the Resilience Commercial Development Team supporting the Gene Therapy Franchise. Dr Jesuraj received her PhD from Washington University in St Louis where she designed Schwann-cell based therapies to regenerate peripheral nerves across acellular nerve grafts. Following her graduate work, she spent 8+ years in various Research roles, including leading an R&D team at Bio-Techne. As an R&D leader, Dr Jesuraj contributed to the successful development and launch of reagents used in immune cell therapy therapies. Dr Jesuraj pursued a career in Product Management where she managed and helped grow Bio-Techne's CDMO business prior to joining Resilience.

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AUTHORSHIP & CONFLICT OF INTEREST

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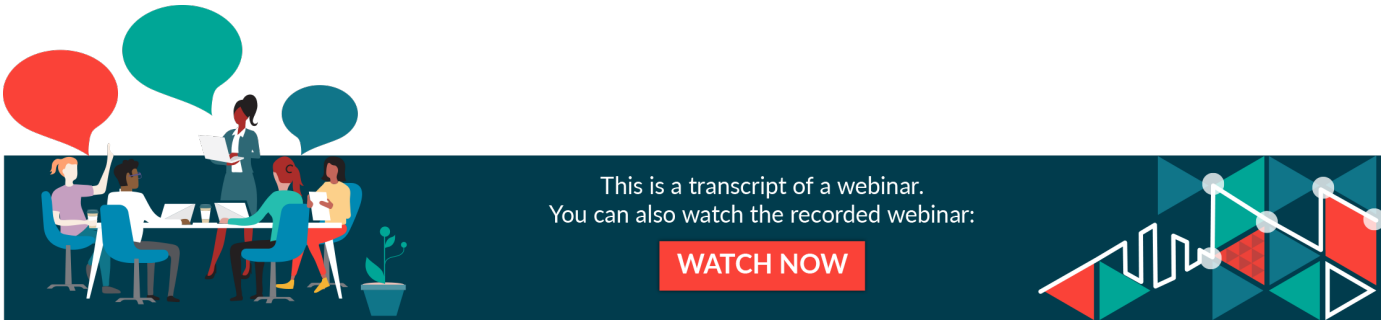

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